Ecological Characterization of Bioluminescence in Mangrove Lagoon, Salt River Bay, St. Croix, USVI

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Introduction

Mangrove Lagoon is a small, man-made embayment (constructed between 1961-1971) on the northeastern side of Salt River Bay, St. Croix, U.S. Virgin Islands (USVI). This shallow lagoon has become a popular tourist attraction over the past decade because of the persistent, brilliant bioluminescence within its waters. The research described in this document is a multi-investigator, multi-disciplinary, and multi-institutional project designed to provide a more detailed description of the physical, chemical, and biological characteristics associated with the bioluminescence phenomenon in Mangrove Lagoon. This study confirms the identification of the bioluminescent dinoflagellate species, demonstrates relationships between water quality parameters and dinoflagellate (as well as all phytoplankton) concentrations, determines which nutrients are potentially limiting the growth of dinoflagellates (and phytoplankton), and maps the spatial distribution, abundance, and a chronology of previous blooms based on preserved cysts within the sediments of the lagoon. Water residence time is approximated based on tidal range and the basin volume.

The study site, Mangrove Lagoon, is found within Salt River Bay National Historical Park and Ecological Preserve (SARI). This National Park is located along the north/central coast of St. Croix (Fig. 1). The Park, co-managed by the National Park Service (NPS) and the Government of the Virgin Islands of the United States (GVI) was created in 1992, to preserve, protect, and interpret nationally significant natural, historical, and cultural resources for the benefit of present and future generations (United States Congress 1992). The 410 ha (1015 acres) of the park encompass a combination of marine, estuarine, and terrestrial habitats including the largest remaining mangrove forest within the US Virgin Islands, coral reefs, seagrass beds, and a submarine canyon.

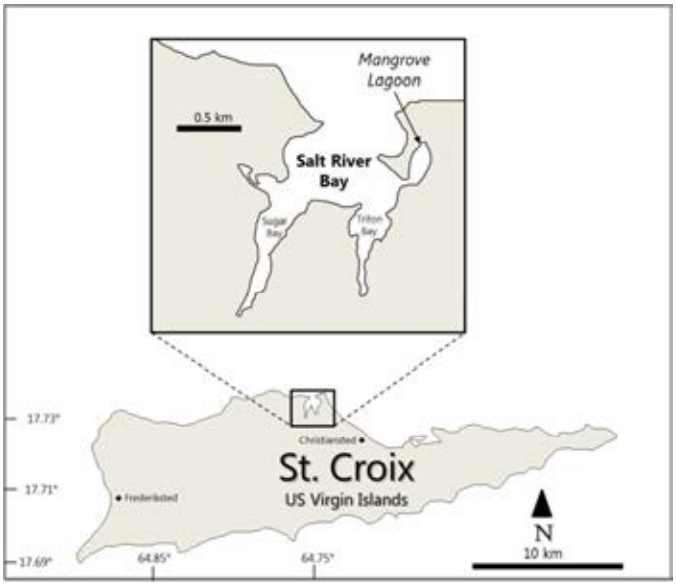

Figure 1. Location map for Salt River Bay and Mangrove Lagoon, St. Croix, U.S. Virgin Islands.

In 1999, NPS, in partnership with the Joint Institute for Caribbean Marine Studies (JICMS), began planning the development of a Marine Research and Education Center (MREC) campus at Salt River Bay. JICMS, a consortium of universities including the University of the Virgin Islands (UVI); University of North Carolina Wilmington (UNCW); Rutgers, the State University of New Jersey; and University of South Carolina (USC) provides a unique opportunity to pair the research and educational missions of the universities with the resources and science-based management needs of the NPS and GVI and house the base of operations within the park itself.

After 17 years, the NPS and GVI established a cooperative agreement to begin collaborative management actions including the development of the east side park access road and the MREC campus (2009). The partners envision the MREC as a small scale development/restoration project that will advance the stewardship of USVI's marine resources, facilitate landscape restoration within the NPS 29.5 ha (73acres) at Hemer's Peninsula, and provide substantial educational and economic benefit to its citizens, local communities, and the general public. The MREC will serve as a center of excellence for marine research and education but also as a model "green campus," relying on renewable energy such as wind and solar power while minimizing impacts to the sensitive habitats such as mangroves, estuary, and adjacent marine areas and avoiding impacts to any of the intact remaining cultural resources.

In 2004, recognizing the benefits of the marine laboratory and the long-term value of locating it in the park, NPS conducted a Feasibility Study, funded by the DOI/Coral Reef Program, to determine if Salt River Bay could support the MREC concept. The study, completed in 2006, found that the federally-owned 29.5 ha site on the east side of the bay was the most feasible location for this project. NPS conducted an Environmental Assessment and a Finding of No Significant Impact (FONSI) was signed in February 2009. The MREC lab/education facility will occupy less than 0.8 ha (2 acres) of a 29.5 ha NPS parcel on Hemer's Peninsula. This parcel of land was extensively altered during the late 1960s to support a proposed hotel resort/marina project that failed. The MREC campus seeks to minimize the amount of land developed for the MREC and to restore the remaining land area to more natural conditions, protect archeological resources, and provide for park visitor enjoyment including trails and coastal experiences. A two- to four-meter deep man-made lagoon is central to the east site and would be the access point to Salt River Bay and coastal studies. Mangrove Lagoon includes a shallow dredged boat channel that opens into the Salt River Bay that is about 300 meters from the primary bay inlet, coral reef shelf, and the open ocean, providing efficient access to the sea from the site of the proposed facility as well as access to high quality sea water just outside the reef of the east side which is of paramount importance for MREC laboratory controlled research into coral reef stressors such as climate change, diseases, and pollution impacts.

Figure 1. Weather station at SARI

In keeping with the mission of the NPS, SARI and university and federal partners have been conducting resource management and research activities to further the goals of the park and inform the MREC campus development. Since 2009 these activities have included demolition and removal of an abandoned commercial structure (hotel) and construction materials, archeological field school to delineate intact cultural resources, shoreline stabilization, evaluation of man-made peninsula, re-establishment of park historic access road, year-long sea water-water quality survey inside/outside the bay, established an automated weather station recording rainfall, wind speed, temperature (Fig. 1), a park-wide vegetation inventory and map, and in 2012 NPS

established a long-term coral monitoring site, planted native plants through USFWS coastal restoration program and conducted control of non-native invasive plants around the mangrove lagoon and peninsula to improve native plant diversity and wildlife habitat.

The Salt River Bay Watershed APC/APR Comprehensive Analytic Study (1993) stated that the bay depends on a combination of winds, currents, and, to a lesser extent, tides to achieve flushing. None of these factors exert very strong influence on the waters of this well-protected, semi-enclosed bay, and flushing capacity is consequently rather poor. Portions of Salt River Bay have been extensively dredged and filled over the past 30 years for a variety of projects including navigation channels, marinas, and boat basins (Island Resources Foundation 1993). The pollution susceptibility of this type of bay is considered to be high, and is discussed in Nichols and Kuo (1979). However, under natural (unpolluted) conditions, restricted bays can be highly productive as nutrients under these conditions have a long residence time. Short-term or temporal changes can often be tolerated by marine biota, but inputs of sediment, nutrients, freshwater, and toxic compounds in the Salt River estuary have long residence times and hence more prolonged effects on biological communities (USVI Govt/DPNR, 1988). Unfortunately, since the drafting of the SARI APC/APR, the bay has continued to suffer from upland erosion from development and land clearing within the watershed, discharges from live-aboard boats, construction of fiberglass boats (west side of the bay), and failed private septic systems which are all having adverse effects on the water quality and turbidity in the bay (Rothenberger et al. 2008, DPNR 2010).

Salt River Bay is comprised of class B waters. Class B waters have been designated "for maintenance and propagation of desirable species of aquatic life (including threatened & endangered species listed, pursuant to section 4 of the federal Endangered Species Act and threatened, endangered and indigenous species listed pursuant Title 12, Chapter 2 of the Virgin Islands Code and for primary contact recreation (swimming, water skiing, etc.)" (DPNR, 2010). Currently, EPA Region 2 has classified Salt River Bay as an impaired body of water. In October 2010, EPA published a list of impaired and threatened waters in the U.S. Virgin Islands that are targeted for the development of future Total Maximum Daily Load (TMDL) limits. On that list, enterococci, fecal coliform, and turbidity were reported impairments listed for Salt River Bay. These impairments were also listed in 2008 and were indicated to be a result of erosion from derelict land, boating on-vessel discharges and residential sources. In 2010, turbidity caused from land development, erosion & sedimentation and urban runoff was added to the list of impairments, all of which are in need of TMDL development. Biochemical oxygen demand (BOD) and sediment oxygen demand (SOD) were also listed as impairments in Salt River Bay during the 1998, 2002, and 2004 listing periods. As a result, a DO TMDL was developed based on dissolved oxygen consumption in the bay (DPNR, 2004). A water quality management program is in place. However TMDL development and then mitigation efforts are needed to meet Class B water quality standards for Salt River Bay. Both NPS and GVI must take actions over the long term to reduce point and non-point sources of pollution into the bay and improve the overall water quality to support the nursery, mangrove, and coral reef ecosystems.

Mangrove Lagoon is located on the east side of Salt River Bay, south of Hemer's Peninsula on St. Croix, Virgin Islands. The oval-shaped Mangrove Lagoon is a small (250 m x 130 m, 3.8 ha), shallow (<4 m) manmade embayment created by a hotel/marina development project in the 1960's-1970's which dredged an existing salt pond connecting a previously enclosed pond to Salt River Bay (Kendall et al. 2005)(Fig. 2). Bioluminescence in the waters of Mangrove Lagoon has become an ecotourism attraction for St. Croix. Over the last 15 years, night kayak tours have been conducted to observe the natural bioluminescence in Mangrove Lagoon. Initially tours were once per month increasing to twice per week 10 years ago and most recently up to 5 times per week (pers. comm., L. Dunton, Anchor Dive).

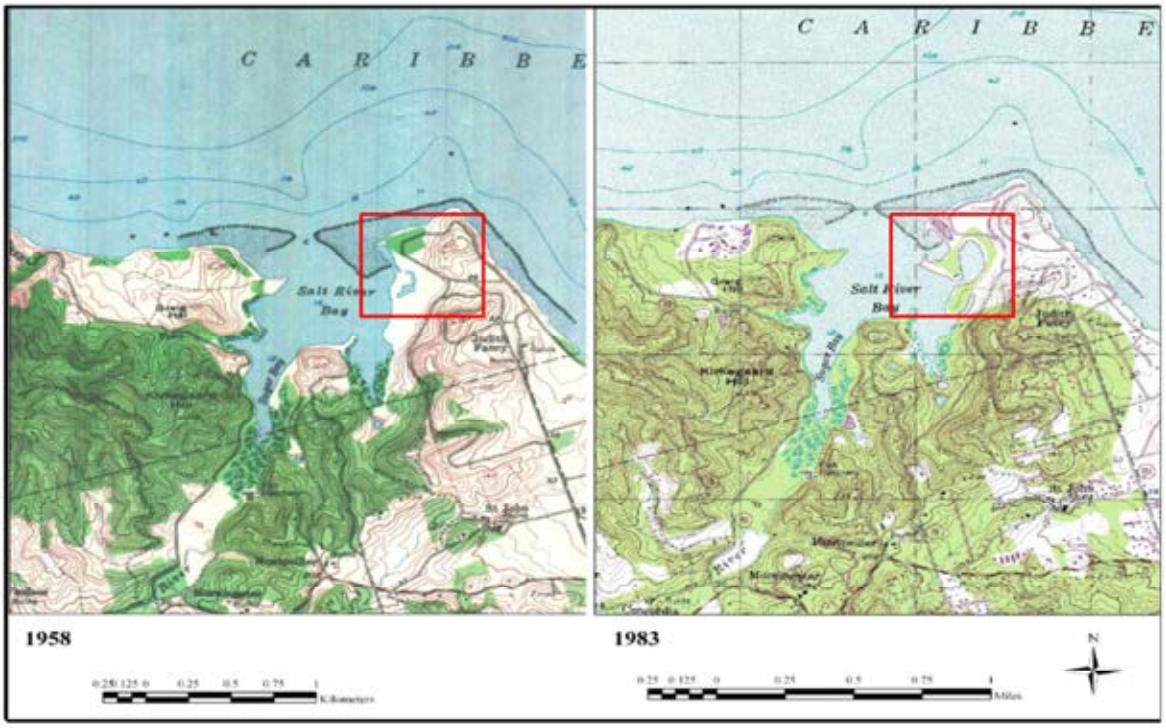

Figure 2. Comparison of topographic maps from 1958 and 1983 showing the construction location of Mangrove Lagoon

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Background: Bioluminescent Dinoflagellates in Caribbean Waters

Biobays

Bioluminescence by dense concentrations of dinoflagellates in enclosed tropical bays results in a phenomenon known as a bioluminescent bay or biobay. There are only fourteen known persistent biobays worldwide and 11 have been reported in the Caribbean region (Table 1). Biobays seem to require specific environmental conditions to persist over time, such as prolonged water retention time, nutrient availability, shallow basin bathymetry, and limited tidal range (U.S Dept. of Interior 1968, Seliger et al. 1970, 1971, Walker 1998, Sastre et al. 2013). Most biobays are surrounded by mangroves which may provide nutrients and dissolved organic matter that is essential for bioluminescent dinoflagellates (U.S Dept. of Interior 1968, Seliger et al. 1971, O'Connell et al. 2007, Viruet 2007).

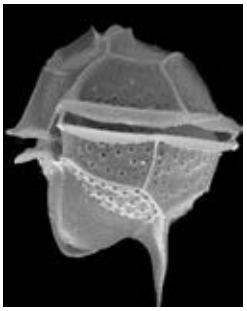

Figure 1. SEM image of *Pyrodinium bahamense* var. *bahamens*e

Pyrodinium

Table 1. Reported locations of biobays in the Caribbean and Gulf of Mexico

Location	Reference
Fire Lake, Nassau, Bahamas	Harvey 1952
Oyster Bay, Jamaica	Seliger & McElroy 1968, Seliger et al. 1970
Tampa Bay, Florida	Steidinger et al. 1980, Phlips et al. 2006, Badylak et al. 2007
Florida Bay, Florida	Phlips & Badylak 1996, Phlips et al. 2006
Indian River Lagoon, Florida	Badylak et al. 2004, Phlips et al. 2006
Bahia Fosforescente, Puerto Rico	Margalef 1961, Seixas 1983, 1988, Walker 1997, Soler-Figueroa 2006
Bahia Monsio José, Puerto Rico	Seixas 1983
Puerto Mosquito, Vieques, Puerto Rico	Walker 1998, Soler-Figueroa 2006
Laguna Joyuda, Puerto Rico	Carvajal-Zamora 1976
Laguna Grande, Fajardo, Puerto Rico	Candelas et al. 1968, Zayas 1979, Sastre et al. 2013, Lane et al. 2013
Mangrove Lagoon, St. Croix, USVI	This report

Pyrodinium bahamense var. *bahamense* is a bioluminescent dinoflagellate common to all biobays in the Caribbean (Steidinger et al. 1980, Steidinger & Tangen 1997, Gasparich 2007). *Pyrodinium* ranges in size from 30 to 60 µm (Gasparich 2007) and has a generation time as long as 3-3.5 days (Seliger et al. 1970). This species is found in warm tropical water (> 20°C), but blooms at temperatures greater than 25°C (Philips et al. 2006). *Pyrodinium* has a wide salinity tolerance ranging from 14 to 46 ppt and may bloom at salinities < 25 (Phlips et al. 2004). In biobays, the highest concentrations of *Pyrodinium* cells are usually found furthest from the mouth of the bay due to a more stable environment and lower flushing rates (Gasparich 2007, Algeo 2008).

Seliger et al. (1971) reported that *P. bahamense* is positively photactic when in tropical sunlight and migrates to depths at dusk, allowing for nutrient uptake from the depths at night. However, *Pyrodinium* is usually seen in high

concentrations in surface waters at night. Soli (1966) found that *Pyrodinium* migrated to the surface around dusk but individuals could be found throughout the water column. Around midnight, the dinoflagellates started their descent to the depths. After sunrise, *Pyrodinium* migrated back to the surface and descended again in the late afternoon, making two cycles in a 24 hour period. Vertical migration may also play a role in dinoflagellate retention in lagoons by allowing cells to avoid being exported from the lagoon by currents and circulation (Anderson & Stolzenbach 1985). *Pyrodinium* has a 'biological clock' resulting in the in ability to produce light during the night. In order for *Pyrodinium* to produce light at night they require a significant amount of light exposure to produce and store energy during the day (Walker 1998).

Dinoflagellates and other phytoplankton in estuarine and coastal systems are usually limited by sunlight or, more commonly, nutrients (Hecky & Kilham 1988, Cloern 1999). Nitrogen and phosphorus are the most common limiting macronutrients for phytoplankton. Phosphorus is usually thought to be the limiting nutrient in tropical, carbonate rich waters. Sediments in the tropics commonly act as a P sink because phosphorus binds to calcium carbonate forming a more stable form called apatite, making it less bioavailable to organisms (Pain 2008). Porewater that is undersaturated with calcite or aragonite would promote phosphorus dissolution, making the sediment a source of phosphorus (Palevsky 2007, Pain 2008). Anoxic conditions in sediments or the overlying waters also promote the export of P from sediments. Commonly, phytoplankton are found to have a C:N:P nutrient ratio of 106:16:1. In environments where nitrogen is limiting the N:P ratio is <16, while phosphorus limited environments have a ratio greater than 16. However, stoichiometric bases for nutrient limitation are frequently flawed and do not always predict nutrient limitation.

In the Indian River Lagoon, Florida, concentrations of *Pyrodinium* were not correlated with phosphorus levels (ranging from 8 to 100 μg l^{-1} or 0.26 to 3.3 μM) (Philips et al. 2006). However, blooming populations were found at dissolved inorganic phosphorus (DIP) levels greater than 300 μg l^{-1} (9.7 μM)(Philips et al. 2006). *Pyrodinium* concentrations, in addition, were not correlated with nitrogen levels but peak population concentrations occurred at 600 μg l^{-1} (43 μM N) dissolved inorganic nitrogen (DIN) (Philips et al. 2006). Phlips et al. (2006) also reported that there was no relationship between *Pyrodinium* abundance and N:P ratios. In the Gulf of California, *Pyrodinium* abundance was positively correlated with salinity, seawater temperature, silicates and ammonium concentrations and negatively correlated with dissolved oxygen (Morquecho et al. 2012)

Under adverse conditions, many dinoflagellate species, including *Pyrodinium*, may form resistant cysts that are eventually deposited in sediments. Villanoy et al. (2006) showed that wind forcing events served to mix surficial sediments up into the water column, allowing the cells to excyst and form dinoflagellate blooms under favorable conditions. Thus strong storms may provide "seed" cysts that take advantage of the higher nutrient concentrations due to runoff.

An important yet mostly unanswered ecological question is "why is *Pyrodinium* abundant in biobays?". Possible explanations include: 1) the tannins and phenols released from the mangroves inhibit the growth of other phytoplankton, 2) *Pyrodinium* produces saxitoxin that inhibits growth of other phytoplankton or reduces mortality due to grazing (Usup & Azanza 1998, Usup et al. 2012), 3) the large size and motility allows *Pyrodinium* to search for and store nutrients, resulting in them out-competing other phytoplankton (Usup & Azanza 1998, Usup et al. 2012), 4) biobays favor flagellated phytoplankton over diatoms due to the low vertical mixing and stability of the water mass, 5) Specific nutrient(s) released by red mangroves are essential for *Pyrodinium* growth and bioluminescence.

Phlips et al. (2006) proposed 3 specific hypotheses for conditions under which *Pyrodinium* may out-compete other species of phytoplankton. (1) the wide salinity tolerance of *Pyrodinium* allows it to out-compete other species with narrow salinity tolerances under conditions of temporally variable salinity regimes, (2) production of toxins may inhibit zooplankton grazing of *Pyrodinium* thereby reducing top-down control of standing crop potential, and (3) other faster growing species may be able to out-compete the slower growing *Pyrodinium* under short water residence times and persistent high nutrient loading rates. Conversely, the large size and motility may allow *Pyrodinium* to search for and store nutrients in stable water columns when nutrient supply is restricted or episodic. Thus long residence times, stable water columns, and variable nutrient concentrations would favor *Pyrodinium* over other phytoplankton species.

Dinoflagellate Bioluminescence

Bioluminescence occurs in fourteen phyla and is common in bacteria, fish, crustaceans, and cnidarians. There are approximately 81 species of bioluminescent dinoflagellates (Marcincko et al. 2013). The dinoflagellate bioluminescent reaction occurs in scintillons, organelles that are part of the vacuolar membrane (Lambert 2006). Protons are released into the scintillon from the vacuole and cause a drop in pH from 8 to 5.7, which facilitate the bioluminescence reaction (Lambert 2006, Smith et al. 2011). The reaction is catalyzed by the enzyme luciferase, which oxidizes luciferin and results in light emission and the by-product oxyluciferase (Lambert 2006, Pain 2008). Bioluminescence is energetically expensive to dinoflagellates and estimated to consume about 255 J mol^{-1} per reaction (Rees et al. 1998). Although costly, dinoflagellates expend energy on bioluminescence at the expense of growth, leading researchers to believe the purpose of bioluminescence is necessary and beneficial for the dinoflagellate.

Dinoflagellate bioluminescence emits light in the range of 474-476 nm as a burst lasting on average 100 msec (Marcinko et al. 2013). The duration, wavelength, and intensity vary among species and depend on the cell's history (Marcinko et al. 2013). Throughout the night, the stored luciferin is depleted and the light emitted will decrease as the night passes (Marcinko et al. 2013). In addition, the amount of irradiance from the previous day can affect the intensity and amount of bioluminescence the following night. Buskey et al. (1994) showed that cultured dinoflagellates held without food were unable to bioluminesce suggesting that the nutritional state of the cell may

affect the ability to emit light. In addition, dinoflagellates were shown to preferentially use energy to bioluminesce rather than to grow. Latz and Jeong (1996) found that *Pyrodinium* cf. *divergens* turned to cannibalism when starved and suggests that dinoflagellates invest heavily in bioluminescence (Marcincko et al. 2013).

There are two main hypotheses for why some species bioluminescence (Harvey 1952). The 'burglar alarm' hypothesis suggests that when a zooplankter is close to phytoplankton, the phytoplankton will bioluminesce, alerting potential predators of the zooplankter (White 1979, Morin 1983, Young 1983). The predator will hopefully eat the zooplankter allowing the dinoflagellate to escape predation. The second hypothesis suggests that light emission at night startles zooplankton, allowing the dinoflagellate to escape (Esaias & Curl 1972, Buskey & Swift 1983, Buskey et al. 1983, Rees et al. 1998). Both these hypotheses are accepted by the scientific community as possible explanations for why bioluminescence occurs, though the exact reason is unknown.

After a series of cloudy days the intensity of bioluminescence produced by dinoflagellates decreases (Walker 1998). Seixas (1988) reported that dinoflagellate population abundance declines when there is high precipitation and high cloud coverage. This was also observed in Bahia Fosforescente, a bay located in Puerto Rico, where the *Pyrodinium* population nearly disappeared after 25.4 cm of rain fell in less than a two week period (Walker 1998). Other observations in Florida have contradicted these findings, with a *Pyrodinium* abundance increase following a major rainfall event (Phlips et al. 2006).

Bioluminescence can be quantified using a bathyphotometer (BP), which measures the amount of light emitted following a mechanical stimulus (Marcincko et al. 2013). The BP can provide estimates of the bioluminescence potential within a known volume of water. However, there are no standards for different types of BPs, and the lack of common units makes comparisons of different types of bioluminescence difficult (Marcincko et al. 2013). Comparisons of values between studies are difficult due to the lack of standardized units and different techniques for sampling. However, measurements are useful for comparisons of relative fluorescence within a habitat.

Importance of Mangroves

Mangroves are extremely important ecosystems that protect coastlines, filter pollutants, and slow excess sediment from entering the bay (Lee 1995, Algeo 2008). Mangroves produce large amounts of organic matter (up to 2,500 mg C m^{-2} d^{-1}) which may be an important source of nutrients for biobays (Lee 1995, Gonneea et al. 2004). Most biobays are surrounded by the the red mangrove, *Rhizophora mangle*. Red mangroves appear to provide nutrients that are essential to the dinoflagellates (Prakash & Rashid 1968, Bernache-Baker 1995, Philips et al. 2006, Trainer 2007); but it is unknown which nutrient or compound is mainly responsible. Mangroves are a significant source of dissolved organic matter (DOM), which can be used not only by the phytoplankton present, but also bacteria. Bacteria absorb these materials and convert them into essential nutrients needed by *Pyrodinium*, such as vitamin B_{12}. Dinoflagellates must acquire vitamin B_{12} from their environment as they cannot produce them on their own (Starr et al. 1957, Burkholder et al. 1967).

Florentine (2007), in an investigation of nutrient budgets for three tropical bays in Puerto Rico, observed plant material accumulation and decomposition differences between seagrass and mangroves, as a possible explanation for the differences in abundances of bioluminescent dinoflagellates. The biobay, Puerto Mosquito, was found to have the lowest carbon isotope ratios and TIC (total inorganic carbon) values suggesting that, relative to the organic matter in the other bays, Puerto Mosquito is dominated by mangrove derived organic matter and the sediment is primarily from a terrestrial source (Florentine 2007). The other two bays are not considered biobays because the *Pyrodinium* population concentration was not high enough to cause the same brightness as Puerto Mosquito. This study supports the hypothesis that organic matter from red mangrove trees may be necessary for *Pyrodinium* to thrive in a biobay.

Ramos et al. (2007) investigated mangrove leaf litter as a source of inorganic nitrogen and phosphorus in a northern Brazil estuary (Ramos et al. 2007). Once leaves fall into the water, chemical and physical reactions occur changing the composition of the leaves. In the first 24 hours, 17 ±2% of the initial dry mass of mangrove leaf litter was leached (Davis et al. 2003). In the first two days of the leaching experiments there was a general increase in TOC, TN, TP in the water surrounding the leaves (Davis et al. 2003). As the leaves fall off the trees into the water, there is an immediate chemical and physical reaction leading to an increase of nutrients in the water system. The more trees surrounding the bay, the more nutrients are made available to be leached into the water. Mangrove leaves also leach a relatively large amount of dissolved organic matter and are an extremely important source of carbon to aquatic ecosystems (Benner et al. 1990a,b). The leaves are a source of labile organic matter in the form of source of sugars, protein, polyphenols, and inorganic nutrients to the surrounding waters (Carlsson & Granéli 1993). Microbial action and the loss of water soluble compounds are primarily responsible for the decomposition of mangrove leaves (Benner et al. 1990b). Mangrove leachate is quickly converted into microbial biomass and moved into the food web (Benner et al. 1986).

Tannins are made by terrestrial plants as a defense mechanism against herbivores and are leached from mangrove leaves. These compounds make water look brown in color and can affect the biogeochemistry in the marine environment by sequestering proteins. Tannins stay in the water for about a day before being transformed either biotically or abiotically (Maie et al. 2008). They are known to positively and negatively affect phytoplankton primary production but the response is species specific (Ake-Castillo & Vazquez 2008). Because tannins are water soluble and reactive, they can inhibit microbial activity and affect the N cycle (Maie et al. 2008). When tannins bind with proteins, they slowly degrade and release N; which may serve as a source of N in the ecosystem (Maie et al. 2008). Mangroves, which are known producers of tannins, may be responsible for inhibiting the growth of some phytoplankton species in biobays, allowing for dinoflagellates to maintain a sustainable biomass.

Mangroves go through a series of predictable color changes. While attached, the leaves are green, then turn yellow and fall, changing from yellow to orange. Yellow leaves collected from the trees were found to have C:H:O:N of

82:10:61:1 while orange leaves collected from the water were found to have C:H:O:N of 94:12:66:1 (Benner et al. 1990b). The various colors leach different nutrients into the water and one color may be more important to biobays.

Saxitoxin

A close relative of *Pyrodinium bahamense* var. *bahamense*, *Pyrodinium bahmanese* var. *compressum*, produces saxitoxin. This species is found in the Indo-Pacific, the Pacific coast of Central America, and recently in the Indian River Lagoon, Florida (Landsberg et al. 2006, Usup et al. 2012). *Pyrodinium bahmanese* var. *compressum* is a significant cause of seafood toxicity, paralytic shellfish poisoning (PSP), in Southeast Asia, Pacific, and Atlantic Coasts along Central America (Usup et al. 2012). In 2006, *Pyrodinium bahamense* var. *bahamense* was reported to produce saxitoxin in the Indian River Lagoon, Florida (Landsberg et al. 2006). However, saxitoxin has not been reported for *P. bahamense* var. *bahamense* in Caribbean biobays. The production of PSP may be an allelopathic mechanism by which *Pyrodinium* inhibits or slows the growth of competing phytoplankton species.

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Salt River Bay showing large terrigenous sediment plume and Mangrove Lagoon (arrow) to east and below grass covered hillside.

PROJECT OBJECTIVES

The purpose of the research described in this report was to perform a comprehensive investigation of the physical, chemical, and biological characteristics associated with the planktonic bioluminescence phenomena in Mangrove Lagoon to determine causal mechanisms and identify key factors necessary for the preservation of bioluminescent activity. Each of the objectives listed below was designed to characterize potentially important ecological/physical factors for dinoflagellate ecology.

The specific objectives (and responsible investigators) were:

I. Confirm the identity of the bioluminescent dinoflagellate(s) and common phytoplankton species in Mangrove Lagoon and an adjacent enclosed pond. (Tomas/UNCW)

II. Collect measurements of basic water quality parameters (e.g., temperature, salinity, dissolved O_2, turbidity, irradiance, nutrients) for comparison with phytoplankton abundances. (Castillo/UVI, Reale-Munroe/UVI, Benitez-Nelson/USC)

III. Map the spatial and temporal distribution of major phytoplankton groups, dinoflagellates, and dormant dinoflagellate cysts in the water column and sediments of Mangrove Lagoon. (Pinckney/USC, Castillo/UVI, Lane/UNCW)

IV. Conduct *in situ* nutrient addition bioassays to determine limiting factors and potential effects of changes in nutrient concentrations on dinoflagellate and phytoplankton abundance. (Pinckney/USC)

V. Estimate water residence time in Mangrove Lagoon. (Pinckney/USC)

VI. Establish an outreach program to inform local citizens and ecotourism industry of research and findings. (Taylor/UVI)

Environmental Planning Requirements and Permits This project was undertaken through Categorical Exclusion (D.O.12, 3.4 E (6) Data collection, research and inventory). USC and UNCW (PI/Pinckney) operated under a Research and Collection Permit from NPS (Study No. SARI 000016, Permit No. SARI-2012-SCI-0001). USC also received a permit to conduct research from Virgin Islands Department of Planning and Natural Resources (Permit No. STX-0007-13). NPS and JICMS universities coordinated research activities with VIDPNR departments and provided results of all data collection and a report for their information on water quality monitoring in Salt River Bay watershed.

Study / Research Methods This project involved a combination of periodic visits by PIs from USC and UNC-W as well as regular weekly sampling by personnel at UVI. The weekly sampling, day-to-day operations, and QA/QC were directed by Dr. Bernard Castillo (UVI), Ky Reale-Munroe (UVI), and Marcia Taylor (UVI/CMES).

UVI undergraduate students collecting water samples in Mangrove Lagoon

Collecting mangrove leaves for nutrient addition bioassays

Table 1. Timeline of events for the study period 1 January 2013 to 31 December 2013.

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Institution and Event description	2013												2014	
	J	F	M	A	M	J	J	A	S	O	N	D	M	
UNCW-Lane: Cyst Collection (7/2012 in ML) (5/2013 in ML + Triton + Sugar Bay)														
Project Kick-off Public Presentation														
USC-Pinckney/Zimberlin, Greenfield: Nutrients & Phytoplankton Bioassays														
USC/UVI: Weekly Sampling of Water Quality & Phytoplankton														
UVI-Reale-Munroe, Castillo, Ruffo: Hourly, Water Chemistry *in situ* Data Collection (YSI sonde)														
UVI-Reale-Munroe, Castillo: Monthly, Day & Night Water Quality Analysis of Nutrients (HACH)														
UNCW-Tomas: Phytoplankton spp./Abundance (3/2013 in ML + SR 1 & 2) (6/2013 in Triton + Sugar Bay)														
UVI-Taylor: Outreach Presentations/Kayak Tours														
USC-Ellis & Wegner: Residence Time/Flushing Rates (AWAC Flow meter)														
UVI- Taylor/Reale-Munroe: Weather Station Data Collection														
Progress/Final Reports														
Public Symposium/ Presentations of Results														

OBJECTIVE I. Confirm the identity of the bioluminescent dinoflagellate(s) and dominant phytoplankton species in Mangrove Lagoon. (Tomas, UNC-W)

METHODS

Water Sampling – Subsurface water was collected from 6 stations within Mangrove Lagoon in March 2013. Sampling was done in the mid-morning until early afternoon. At each of the water sample locations we deployed a screen collector for benthic phytoplankton samples. These were left in place for approximately 24 hours when they were retrieved and returned to the lab or processing and initial examination. These screen samples and the whole water samples were observed and either packaged for shipment back to UNCW or were preserved with Lugol's iodine for further examination. Thus 6 water samples and 6 screen samples were collected for later study. Two net tows were taken in the Lagoon for examination of the dominant larger phytoplankton.

Net sampling for phytoplankton in Mangrove Lagoon.

One additional area was sampled. This was the isolated "salt pond" just north west of the Mangrove Lagoon. This pond was accessible only by foot and through difficult mangrove thicket. A water sample was taken there along with a small net tow and used for examination of the community and for single cell isolations. A night-time sampling of Mangrove Lagoon was done by kayak where a net tow and surface sample were taken. These were returned to the lab and examined the next day.

RESULTS

While these studies (particularly the culture component) are ongoing, some observations were made and can be reported. The Lagoon area had a mixed population consisting of primarily of diatoms and dinoflagellates (mostly photosynthetic) with a number of smaller heterotrophic flagellates. Two putative bioluminescent dinoflagellates were observed. The first, *Pyrodinium bahamense* var. *bahamense* was seen in the Lagoon area and from the limited samples of the Salt Pond (Fig. 1). Surprisingly, the densities were not excessive and normally varied between 10^3 – 10^5 cells l^{-1}. The higher concentrations were found in the night-time sampling and in the surface of the Mangrove Lagoon. This species was rare in Triton and Sugar bays.

The putative bioluminescent species, which was more prolific and widely spread, was one we termed "*Alexandrium*-like" species. We will not know the exact identification of this species until we have stable permanent cultures that we can prepare for SEM microscopy. This *Alexandrium*-like species was prolific particularly in the Mangrove Lagoon area and was one of two dominant species of the "isolated pond". It was also found in Triton and Sugar bays. Like

Pyrodinium, the genus *Alexandrium* is noted for having bioluminescent members many of which produce saxitoxins. Abundances of these cells often exceeded those of *Pyrodinium* and were commonly found in the $10^5 – 10^6$ cells l^{-1}. The higher concentration was recorded for the "isolated pond" but this species was common throughout the lagoon. Other dinoflagellates present in lower numbers included *Ceratium fusus, C. furca, C. lineata, Prorocentrum emarginatum, Prorocentrum micans* and *Protoperidinium quinquicorni*. Relatively few diatoms were present including several species of the genus *Skeletonema*, and a significant number of benthic pennate species which were present in large numbers (>10^5 cells/L) in the "Salt Pond". The most common diatom in the salt pond was *Pleurosigma sp*. that far outnumbered all other diatoms in the pond. The heterotrophic dinoflagellate species *Oxyrrhis marina* was found at all stations.

Figure 1. Location of phytoplankton sampling sites in Salt River Bay

The screen samplers gave particularly interesting results. Here many benthic dinoflagellates were observed consisting primarily of *Prorocentrum* species and *Amphidinium* species. The screen kept near the Crescent Bay reef contained species typically found on or near reef areas. These included species of *Gambierdiscus* (probably *carabeus*), *Coolia* spp, *Ostreopsis* spp., *Prorocentrum belizeanum, P. hoffmannanum, Amphidinium carterae and A. gibbosum*. It should be noted that all these species are associated with ciguatera or tropical fish poisoning for future reference. As these species are indigenous to coral reef areas, it does not mean that there is a ciguatera problem but should serve to inform physicians if human symptoms should appear.

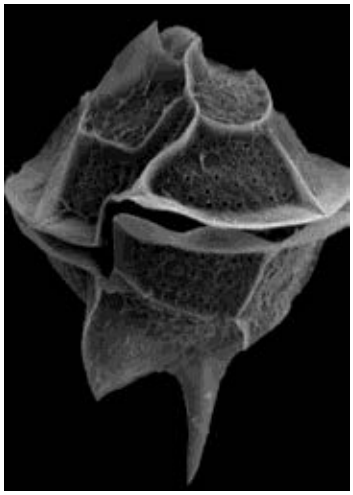
Culture attempts:

From the live samples taken in St. Croix during our March sampling, we presently have a number of cultures in various stages of preparation. These are established all from single cell isolates and placed into media having a salinity of 39 and kept at 25° C. The following is a list of the incipient cultures:

Pryodinium bahamense var. bahamense	6 cultures presently live (slow growing)
Gymnodinium instriatum	2 cultures
Alexandrium spp.	12 cultures
Gambierdiscus sp.	6 cultures
Gymnodinium X1	2 cultures
Gymnodinium X2	4 cultures
Coolia sp.	1 culture

Several other unknowns are in the process of growing. All will be prepared for proper identification using conventional phase contrast microscopy and Scanning Electron Microscopy. Work on these and preserved samples is ongoing.

Alexandrium sp.



Pyrodinium bahamense var. *bahamense*

Water collection in Mangrove Lagoon

OBJECTIVE II. Collect measurements of basic water quality parameters (e.g., temperature, salinity, dissolved O_2, turbidity, pH, irradiance, dissolved nutrients) for comparison with phytoplankton abundances (Castillo, Reale-Munroe, Benitez-Nelson, Pinckney, Taylor)

METHODS

A YSI 6920 water quality monitoring system (sonde) was deployed in Mangrove Lagoon for long-term, *in situ* monitoring within the bay. The sonde recorded salinity, temperature, pH, and dissolved oxygen (DO) at hourly intervals. Data were collected from the sonde and downloaded approximately twice per month. Sonde sensors were cleaned and calibrated approximately twice a month to minimize the effects of fouling.

A HACH 3900 spectrophotometer was used for field measurements to assess temporal and spatial distributions of total nitrogen, total organic carbon, total phosphorous, and nitrate concentrations throughout the lagoon. Collected samples were analyzed using EPA approved, accepted, or equivalent methodologies outlined in each respective HACH reagent protocol. Samples analyzed using the HACH 3900 were collected once a month.

Weekly water samples were collected using a 1 m integrated water column sampler (surface waters) and a horizontal Niskin bottle (0.5 m from the bottom) from 5 fixed locations within Mangrove Lagoon (Fig. II.1). The samples were analyzed for dissolved nutrient concentrations and phytoplankton community composition/biomass. Measured nutrients included nitrate, nitrite, ammonium, orthophosphate, and dissolved organic phosphorus. Water chemistry samples were filtered through sterile 0.45 µm pore size cellulose acetate membrane syringe filters and immediately frozen for later analysis. The concentrations of dissolved inorganic N (NO_2^- + NO_3^-, NH_4^+) were determined using a Lachat Quickchem 8500T autoanalyzer according to the wet chemical methods of Grasshoff et al. (1983), Johnson & Petty (1983), and Zimmerman & Keefe (1991). Samples for soluble reactive phosphorus (SRP) were measured using a modified method of Koroleff (1983). Total dissolved P concentrations, and hence DOP by difference (TDP-SRP = DOP) were measured using a modified method of Monaghan & Ruttenberg (1999). Filtered water samples (ca. 50 ml each) were collected and stored frozen at UVI and later shipped to USC for analysis.

Water sampling in Mangrove Lagoon

Vertical profiles of irradiance at 0.5 m intervals were obtained using a Li-Cor LI192SA spherical PAR sensor. A YSI 6600 sonde was used for measuring the vertical distributions of salinity, pH, dissolved O_2, and chlorophyll fluorescence at each of the stations in the lagoon.

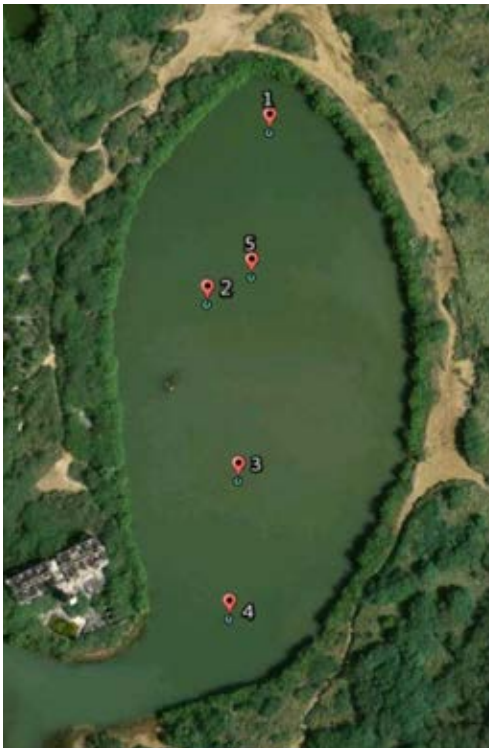


Figure II.1. Location map for sampling stations in Mangrove Lagoon. The *in situ* continuous monitoring sonde was positioned at Station 5.

RESULTS

The YSI 6920 sonde was deployed at a depth of 1.0 m at station 5 (ca. 185 m north of the lagoon inlet) for biweekly intervals during February through January 2014. Water temperature exhibited a relatively normal seasonal pattern with several 2 - 3° cooling events that were correlated with major rain events (Fig. II.2). Rainfall showed a typical dry season pattern from February to April followed by wet summer and fall months. Salinities were highest during the dry season and declined with the cumulative rainfall in the fall months. The salinity record also showed 3-4 periods of rapid freshening that were correlated with periods of high rainfall. However, rapid salinity reductions did not always correspond to rainfall events. Dissolved oxygen concentrations followed a normal diel pattern and rarely dropped below 4 mg l^{-1} (60% saturation). The EPA TMDL endpoint for dissolved oxygen in Class B waters is 5.5 mg l^{-1}. Peaks in turbidity were usually associated with rainfall events and may be attributed to watershed runoff or deep mixing in the lagoon. However, fouling of the turbidity sensor during deployment leads to some uncertainty in the measurements.

Frequency distributions for the continuous data collected during the study period were calculated based on the hourly measurements of pH, DO, temperature, and salinity (Fig II.3). These distributions illustrate the range of values and degree of variation for each of the measured variables. Descriptive statistics for the pooled data for each of the variables are listed in Table II.1.

Table II.1. Summary descriptive statistics for *in situ* continuous sonde measurements in Mangrove Lagoon.

Parameter	Mean	Standard Deviation	Minimum	Median	Maximum
pH	7.8	0.10	7.4	7.8	8.0
DO (mg l^{-1})	5.51	0.53	2.04	5.55	7.03
Temperature (°C)	28.8	1.8	24.2	29.2	33.9
Salinity (psu)	36.90	2.01	28.98	37.38	40.76

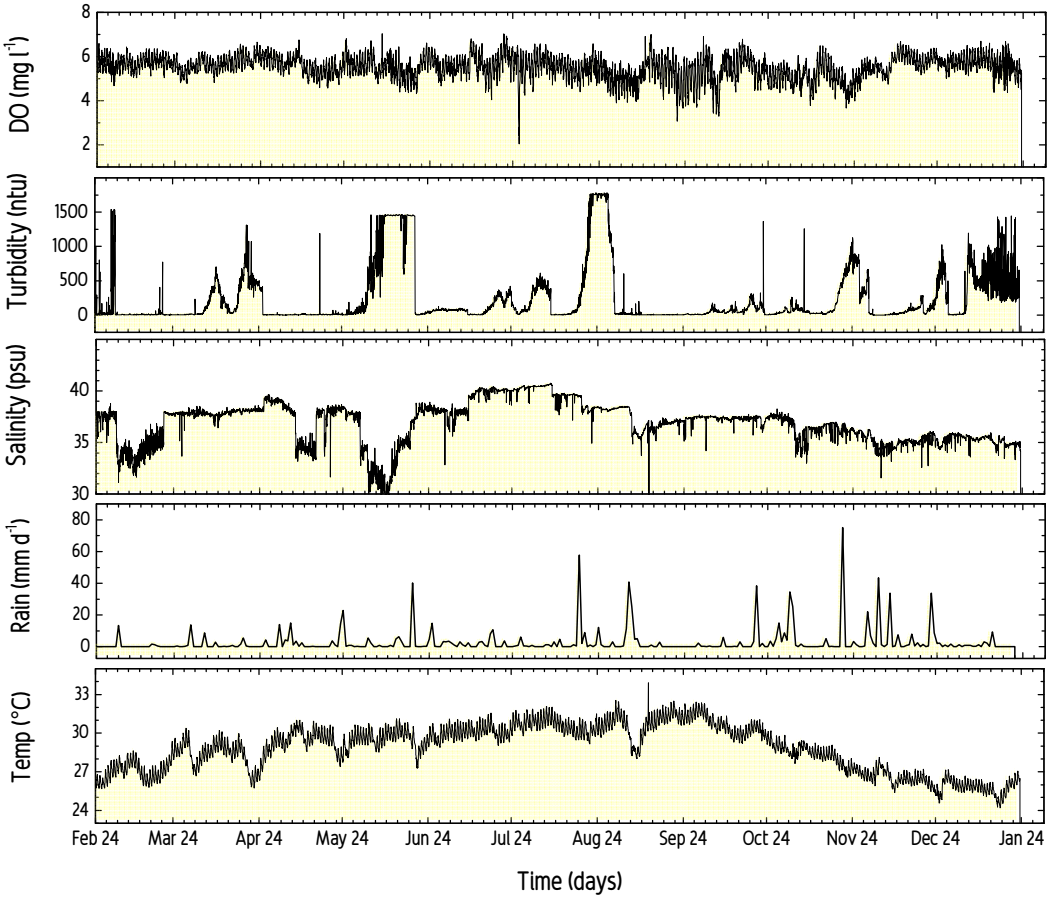


Figure II.2. Temperature, daily precipitation, salinity, turbidity, and dissolved oxygen concentration records from the *in situ* continuous water quality monitor (YSI Sonde) located at Station 5, 185 m north of the entrance to Mangrove Lagoon.

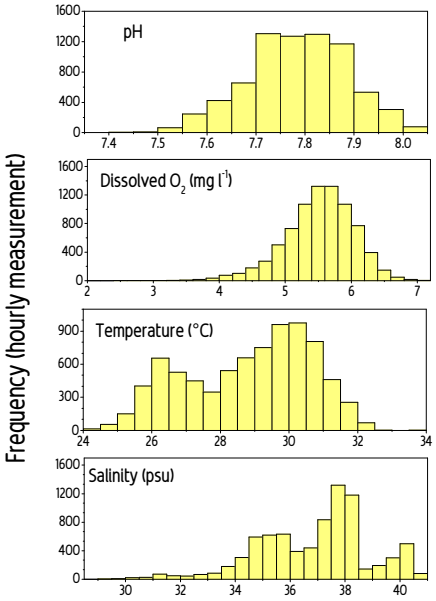


Figure II.3. Frequency distributions of hourly sonde measurements for pH, DO, temperature, and salinity in Mangrove Lagoon.

Nutrient data, based on the HACH methods, were obtained for station 5 at ca. monthly intervals from March 2013 to January 2014. Surface and bottom, day and night samples were pooled for analysis (Fig. II.4). Nitrate showed a general increase over the time period, phosphate and total N had two peaks in concentrations, and total organic carbon had a single peak in August-September 2013. Nutrient data, obtained by wet chemistry methods, were pooled for all 5 stations for each sampling date. Nutrient concentrations were relatively low and variable over the study period (Fig. II.5, II.6, Table II.2, II.3). Total dissolved inorganic nitrogen (DIN; NO_2^- + NO_3^- + NH_4^+) reached a maximum of ca. 8 µM N in April and declined to values as low as ca. 1 µM N in November. The record shows periodic small increases in DIN that appear to be correlated with rainfall events. Ammonium (NH_4^+) was the major contributor to the total DIN in Mangrove Lagoon. Dissolved inorganic phosphate (P; PO_4^{3-}) concentrations ranged

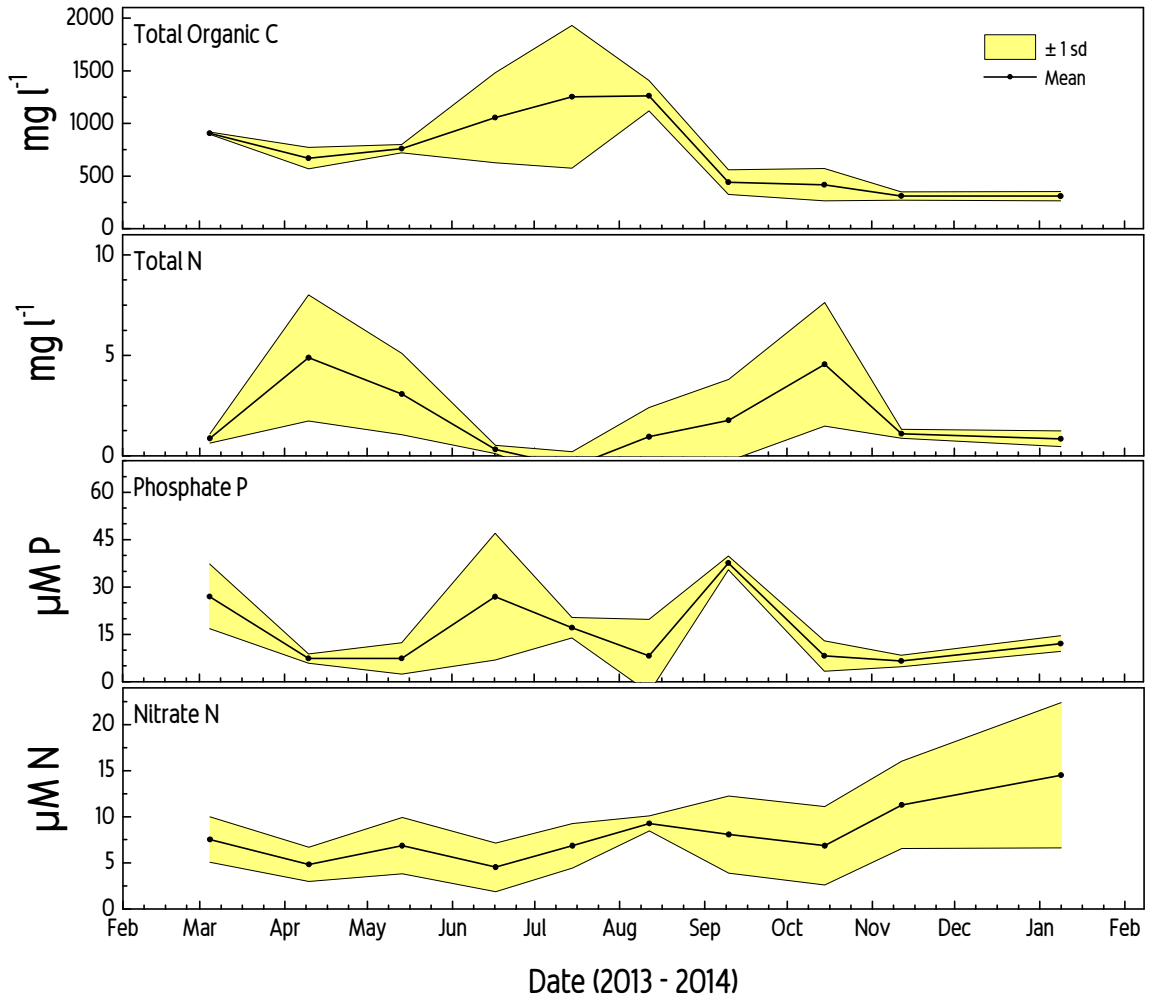


Figure II.4. Nutrient concentrations for station 5 obtained using HACH methods. The colored band indicates ± 1 standard deviation.

from ca. 2 to 0.25 µM P, with the highest concentrations occurring in May and June. Both DIN and P concentrations showed a notable increase in late November along with a major rainfall event. The DIN/P ratio also declined from 5 to 3, with a marked increase in late November. Based on a Redfield Ratio of 16:1, phytoplankton production appeared to be N-limited over the duration of the study. Dissolved organic phosphorus (DOP) concentrations were higher than or equal to soluble reactive phosphorus (SRP). DOP may provide an additional source of P for phytoplankton with the ability to use alkaline phosphatase.

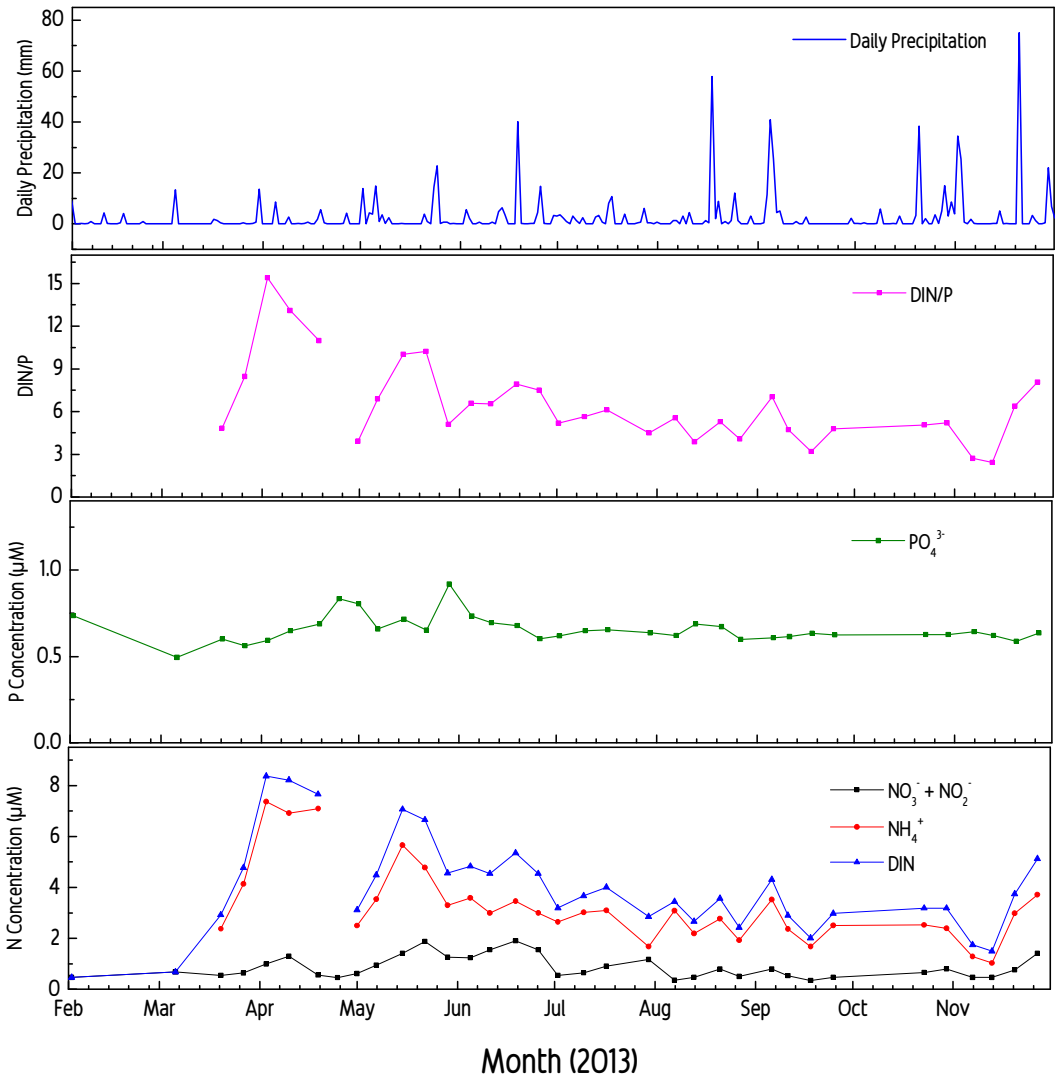


Figure II.5. Average nutrient concentrations and daily precipitation in Mangrove Lagoon obtained using wet chemistry methods.

Table II.2. Summary descriptive statistics for wet chemistry nutrient measurements in Mangrove Lagoon.

Parameter	Mean	Standard Deviation	Minimum	Median	Maximum
Nitrate + Nitrite (µM)	0.86	0.43	0.34	0.66	1.90
Orthophosphate (µM)	0.66	0.08	0.49	0.64	0.92
Ammonium (µM)	3.28	1.57	1.03	3.00	7.37
Dissolved Inorganic N (µM)	3.96	1.94	0.46	3.62	8.37
N to P Ratio	6.5	2.9	2.4	5.6	15.4

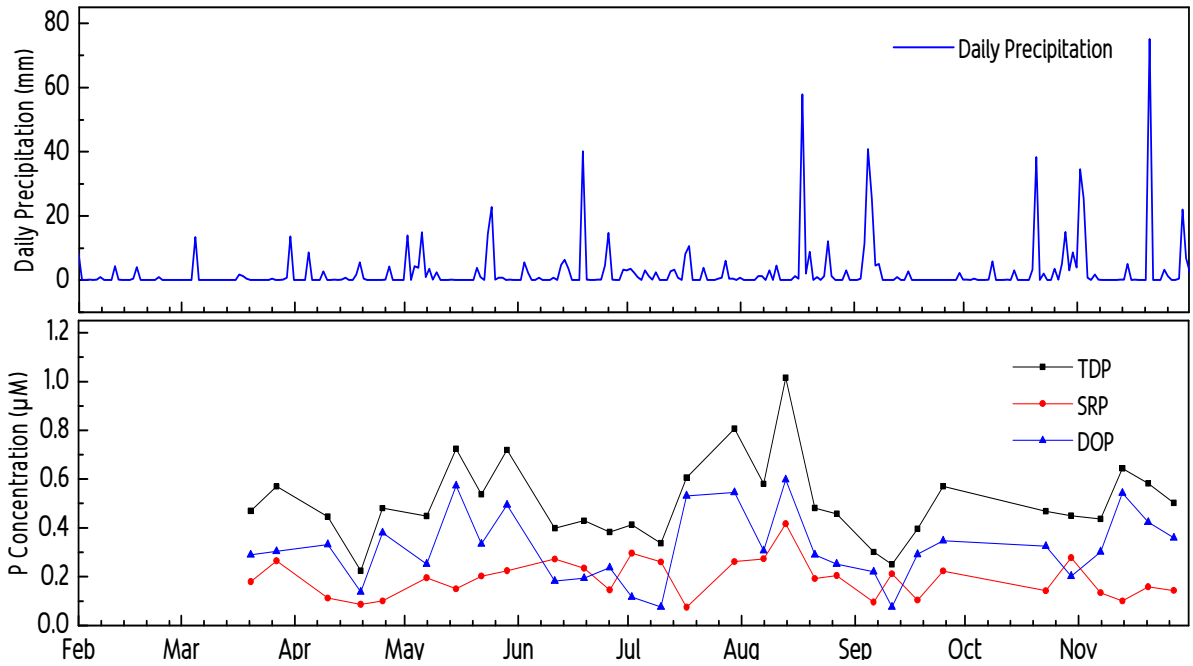


Figure II.6. Average phosphorus nutrient concentrations and daily precipitation in Mangrove Lagoon obtained using wet chemistry methods. TDP = total dissolved phosphorus, SRP = soluble reactive phosphorus, DOP = dissolved organic phosphorus

Table II.3. Summary descriptive statistics for wet chemistry nutrient measurements in Mangrove Lagoon.

Parameter	Mean	Standard Deviation	Minimum	Median	Maximum
Total Dissolved Phosphorus (TDP)	0.50	0.16	0.22	0.47	1.02
Soluble Reactive Phosphorus (SRP)	0.19	0.08	0.08	0.19	0.42
Dissolved Organic Phosphorus (DOP)	0.32	0.14	0.08	0.30	0.60

Vertical profiles of irradiance (PAR) at each station in the lagoon were obtained on 09 January 2013 (Fig. II.7). Using the pooled data, the diffuse attenuation coefficient (k_d) was estimated to be 0.90 ± 0.23 (1 sd). Even though the waters were unusually turbid on this date due to strong winds, the sediment surface at the bottom of the lagoon received sufficient light to support benthic photosynthesis.

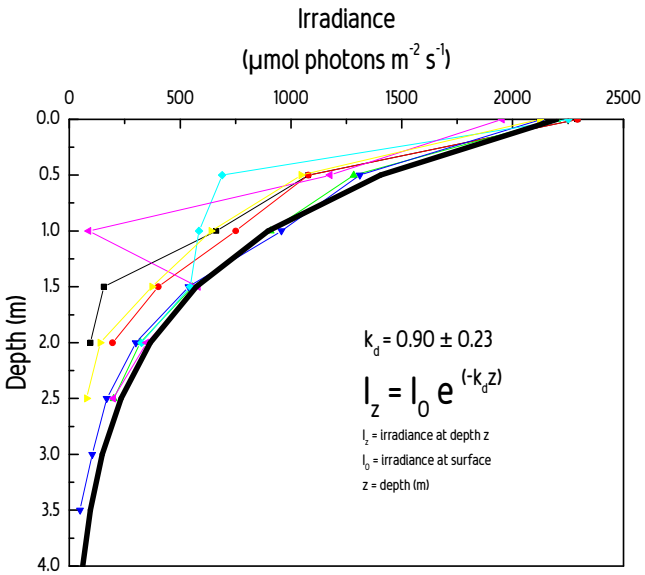


Figure II.7. Results of 7 vertical profiles of irradiance (PAR) on 09 January 2013. Data were fitted to calculate the diffuse attenuation coefficient (k_d).

Salinity, pH, and dissolved O_2 profiles were homogeneous from surface to bottom (data not shown) and suggest a well-mixed water column in Mangrove Lagoon. Profiles of chlorophyll *a* (chl *a*) fluorescence were obtained on 15 May at each of the sampling stations in the lagoon (Fig. II.8). Additional vertical profiles were performed at ca. monthly intervals at Station 5 (Fig. II.9). A comparison of day and night profiles suggests higher chl *a* in the bottom waters during the daytime and a more homogeneous distribution at night. These data show diel phytoplankton vertical migration and negative phototaxis for at least part of the community (ca. 25%).

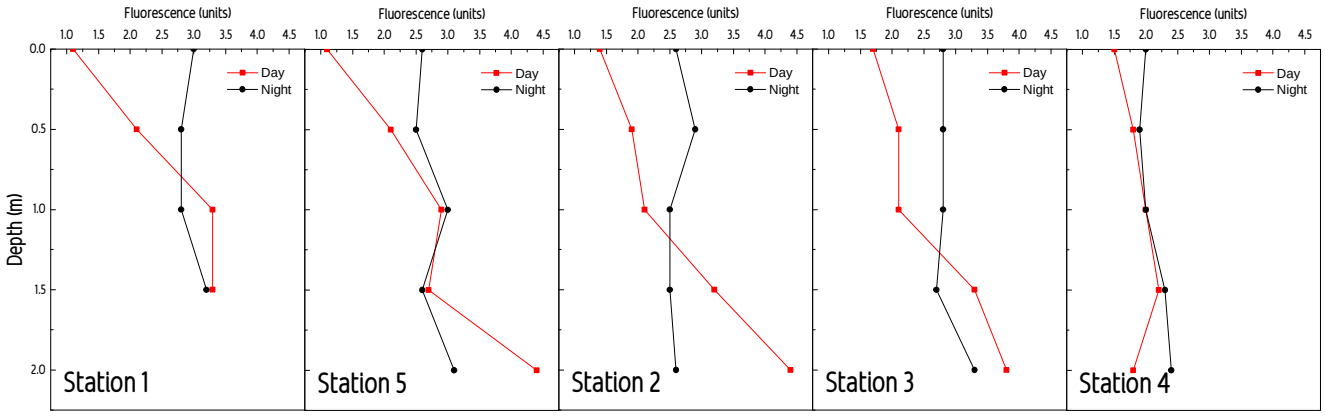


Figure II.8. Vertical profiles of chl *a* fluorescence obtained on 15 May 2013 at each of the sampling stations in Mangrove Lagoon.

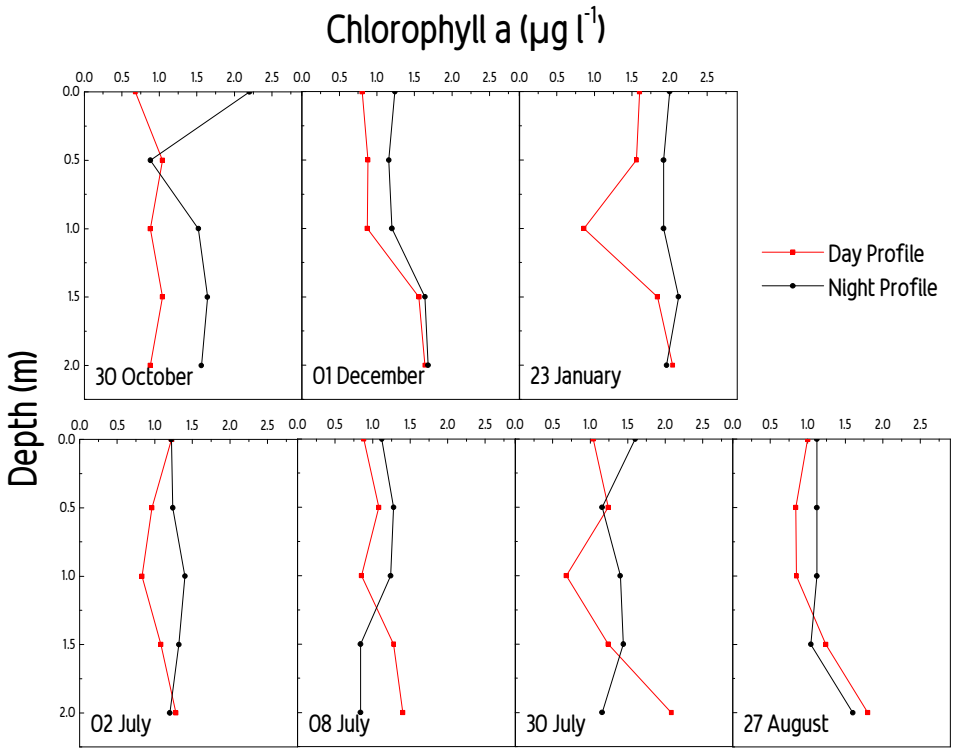


Figure II.9. Vertical profiles of chl *a* obtained at Station 5 for multiple dates in 2013-14 at each of the sampling stations in

SUMMARY

The continuous monitor record showed a typical dry/wet season pattern, with fairly rapid reductions in salinity and increases in turbidity following most rainfall events during the 2012 – 2013 sampling period. Nutrient (N and P) concentrations were generally low, less than 10 µm N and 2 µM P, during the study period and were highest during April – July. The HACH nutrient methods overestimated N and P concentrations relative to standard wet chemistry methods, but both data sets showed similar trends in nutrient concentrations. There was a notable increase in both N and P in late November. The majority of the bioavailable N was in the form of ammonium (NH_4^+) which suggests a benthic origin rather than runoff. Mixing events in the lagoon, due to storms or strong winds, may resuspend sediments and nutrients in the water column.

The contribution of rainfall events to nutrient inputs into Mangrove Lagoon may be influenced by the frequency and duration between rain events. For example, rain following drought periods may not result in runoff because rainwater is absorbed by soils in the watershed. Once the soils are saturated by several consecutive rain events, the runoff may result in nutrient loading to Salt River Bay.

The Redfield Ratio for DIN:P was always less than 16, which suggests N-limitation for phytoplankton growth. Measurements of light attenuation in the lagoon were limited to one visit, but even under turbid conditions, as much as 10-20% of surface irradiance reached the bottom sediments in the lagoon. This is sufficient sunlight to support photosynthesis by benthic microalgae and demersal phytoplankton. Based on the results of profiles with the YSI Sonde, the waters of the lagoon appear to be homogeneous from top to bottom with respect to temperature, salinity, and dissolved O_2. Vertical profiles of chl *a* fluorescence during day and night suggest that a portion of the phytoplankton community (ca. 25%) undergoes migration from bottom to surface waters during nighttime and this behavior occurs over much of the lagoon.

Collectively these results suggest that Mangrove lagoon is a high salinity embayment with low concentrations of inorganic nutrients and relatively low concentrations of dissolved oxygen. The waters of the lagoon can experience rapid changes in salinity (ca. 5 psu) following some rainfall events. Temperature and salinity are well within the reported optimum range for blooms of *Pyrodinium bahamense* (Phlips et al. 2006). Ammonium (NH_4^+) is the predominant form of inorganic N, suggesting a benthic source for most of the N supporting phytoplankton growth. The N:P ratios suggest that phytoplankton production should be N-limited even though the concentrations of dissolved inorganic phosphorus (DIP) are very low (< 1 µM) in the lagoon. The waters of the lagoon satisfy the EPA criteria for Class B waters with respect to dissolved oxygen and total phosphorus concentrations.

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OBJECTIVE III. (A)Map the spatial and temporal distribution of major phytoplankton groups, dinoflagellates, and (B) dormant dinoflagellate cysts in sediments of Mangrove Lagoon (Pinckney, Castillo, Lane, Reidhaar)

(A) Map the spatial and temporal distribution of major phytoplankton groups and dinoflagellates (Pinckney)

METHODS

Weekly water samples (February to November) were collected using a 1 m integrated water column sampler (surface) and a horizontal Niskin bottle (0.5 m from the bottom) from 5 locations within Mangrove Lagoon to determine phytoplankton community composition (Fig. III.A.1). Collected water (ca. 100 - 500 ml) was filtered onto 25 mm dia. Whatman GF/F glass fiber filters under a gentle vacuum. Filters were stored frozen at -80° C and shipped quarterly to USC (Pinckney) for analyses. Photopigments were analyzed using high performance liquid chromatography (HPLC). Filters were first lyophilized for 20-24 h, then extracted in 600-750 µL 90% aqueous acetone at -20°C for 18-22 h. The synthetic carotenoid pigment β-apo-8'-carotenal (Sigma) was used as an internal standard. Filtered extracts (250 µl) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing) and a polymeric (Vydac 201TP54, 0.46 × 25 cm, 5 µm packing) reverse-

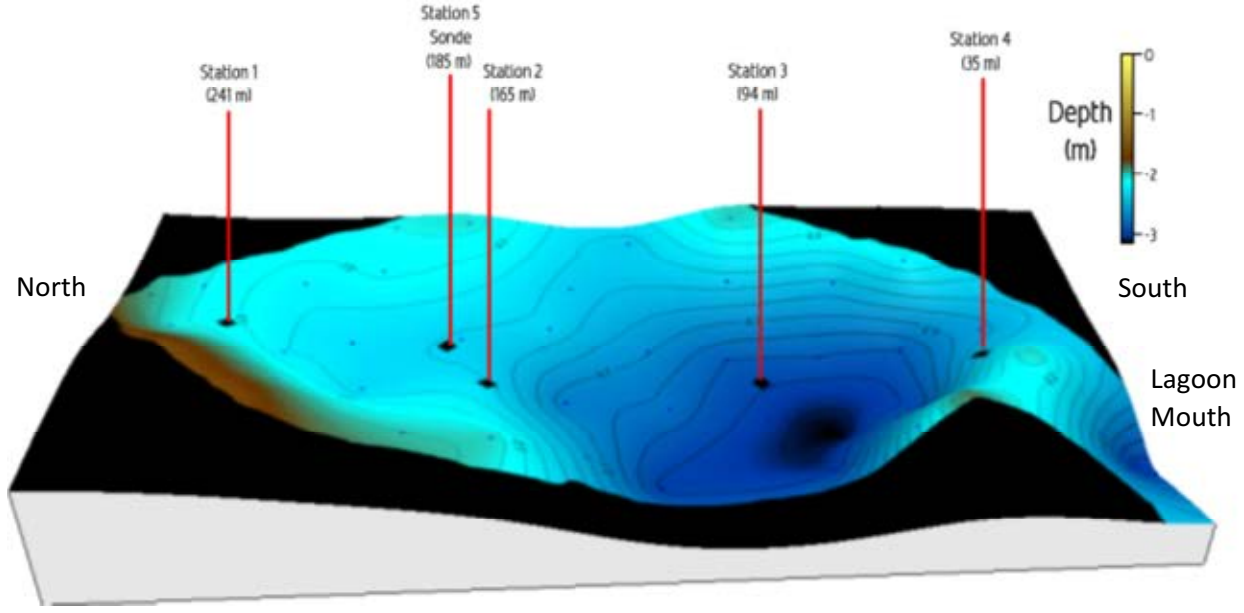

Figure III.A.1. Locations of phytoplankton sampling stations in Mangrove Lagoon. Numbers in parentheses indicate distance from the mouth of the lagoon and these distances are used for the spatiotemporal plots below.

phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol: 20% 0.5 M ammonium acetate) and solvent B (80% methanol: 20% acetone) was the mobile phase (Pinckney et al. 2001). Absorption spectra and chromatograms (440 ± 4 nm) were obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark).

Pigment concentrations were further analyzed using ChemTax (v. 1.95) to determine the relative abundances of major phytoplankton groups (Pinckney et al. 2001, Lewitus et al. 2005, Higgins et al. 2011). The major phytoplankton groups used for ChemTax categories were based on qualitative microscopic examinations of water from the bioassays. The initial pigment ratio matrix used for this analysis was derived from Mackey et al. (1996). The convergence procedure outlined by Latasa (2007) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed values. A two-step cluster analysis procedure based on log-likelihood distance measures of 19 photopigment variables was used to define homogeneous groups for separate ChemTax analyses (SPSS v. 21). The cluster analysis grouped the samples into 1 cluster consisting of 357 samples. The data were analyzed using a single analysis bin in ChemTax to provide estimates of the relative abundances of 8 algal groups. A final root mean square error (RMS Error) of 0.085 was achieved after 15 separate runs. Total chlorophyll *a* was used as a proxy for phytoplankton biomass (i.e., the sum of all phytoplankton groups).

RESULTS

The data for all dates, depths, and stations were pooled to calculate summary statistics for phytoplankton concentrations over the study period (Table III.A.1).

Table III.A.1. Summary statistics for phytoplankton groups. Data were pooled for all depths and dates. Units are µg chl *a* l^{-1}.

Parameter	Mean	Standard Deviation	Minimum	Median	Maximum
Total Chl *a* (all groups)	3.70	3.28	0.23	2.79	27.87
Chlorophytes	0.01	0.01	0.00	0.01	0.05
Cryptophytes	0.05	0.04	0.00	0.05	0.21
Cyanobacteria	0.83	0.40	0.00	0.85	2.23
Diatoms	1.35	1.34	0.00	1.00	10.90
Dinoflagellates	1.32	2.50	0.00	0.45	18.13
Haptophytes 3	0.04	0.03	0.00	0.03	0.19
Haptophytes 4	0.08	0.26	0.00	0.00	2.33
Prasinophytes	0.01	0.02	0.00	0.00	0.19

The spatial and temporal distributions of the total phytoplankton community and the 8 individual algal groups are summarized in Figs. III.A.2-10. The spatiotemporal plots suggest phytoplankton "blooms" occurred in mid-May and September – November. Diatoms and dinoflagellates were the primary groups responsible for the blooms, with a larger contribution of dinoflagellates in the fall period (Fig. III.A.11). There were two diatom blooms, one in the spring (March-April) and late summer (August). Dinoflagellates also exhibited 3 distinct concentration peaks (i.e., "blooms") in August, September, and November. Cyanobacteria concentrations were higher during the warmer summer and early fall months (June – late October). Group 4 haptophytes is a mixed group of haptophytes that contain the accessory pigments 19' butanoyloxyfucoxanthin and 19' hexanoyloxyfucoxanthin. The most common algal group in this category is the prymnesiophytes. Haptophytes group 4 (mostly prymnesiophytes) reached a peak abundance in the bottom waters from September through November. Concentrations of all algal groups were higher in the fall (August – December). Although the blooms were widely dispersed, the persistence of the blooms appeared higher at station 3, near the center of the lagoon. A comparison of the paired surface and bottom concentrations for the groups showed that the concentrations were higher in the bottom water samples for all groups except the prymnesiophytes (paired t-test, $p < 0.01$). The contribution of dinoflagellates to the total phytoplankton community was ca. 10-20% from February to June, then gradually increased to values as high as 90% in late November (Fig. III.A.11).

Plots for the pooled data (all stations, surface and bottom) for total phytoplankton for each sampling date illustrates the bloom periods as well as a large increase in the variability of concentrations in the fall period (Fig. III.A.12). Dinoflagellates also showed a similar increase in both total concentration as well as variability in concentrations for the 5 sampling locations. The x-axis in the plots represents time and the y-axis shows the location in the lagoon. The colors indicate phytoplankton concentrations. The plots show how the distribution and abundance of phytoplankton change over time at different locations within Mangrove Lagoon.

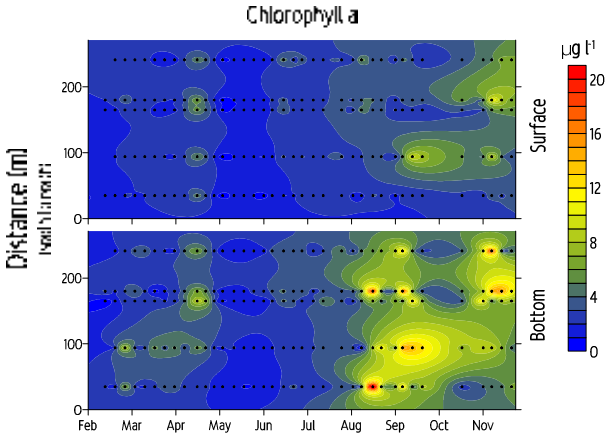

Figure III.A.2. Spatiotemporal contour plot of chlorophyll *a*, an indicator of total phytoplankton concentrations.

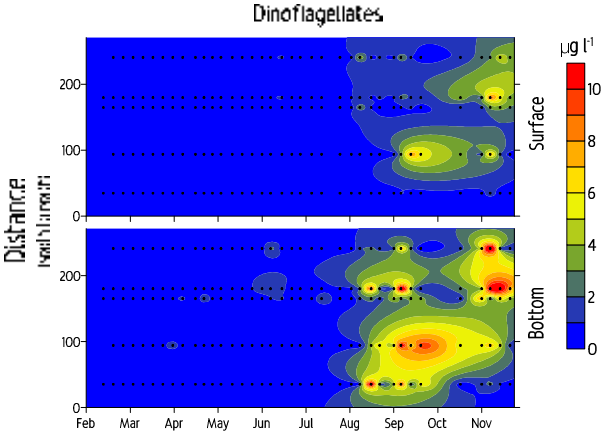

Figure III.A.3. Spatiotemporal contour plot for dinoflagellates.

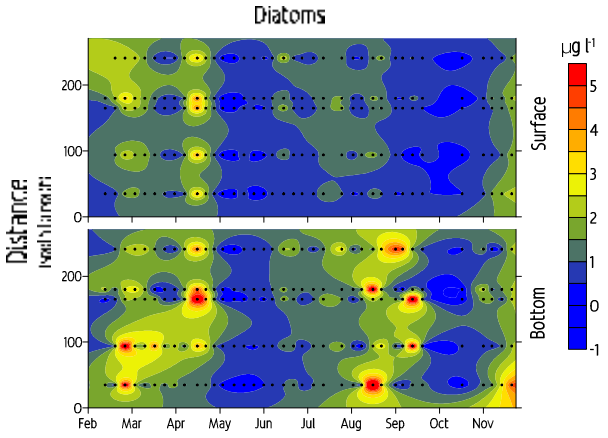

Figure III.A.4. Spatiotemporal contour plot for diatoms.

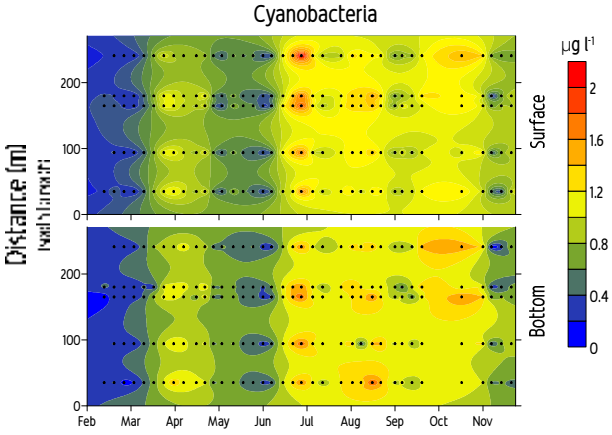

Figure III.A.5. Spatiotemporal contour plot for cyanobacteria.

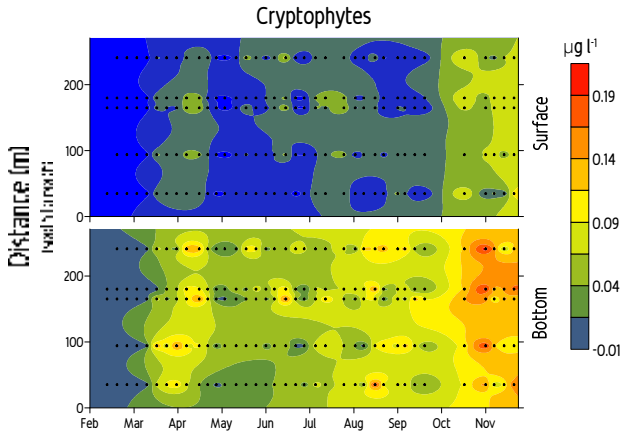

Figure III.A.6. Spatiotemporal contour plot for cryptophytes.

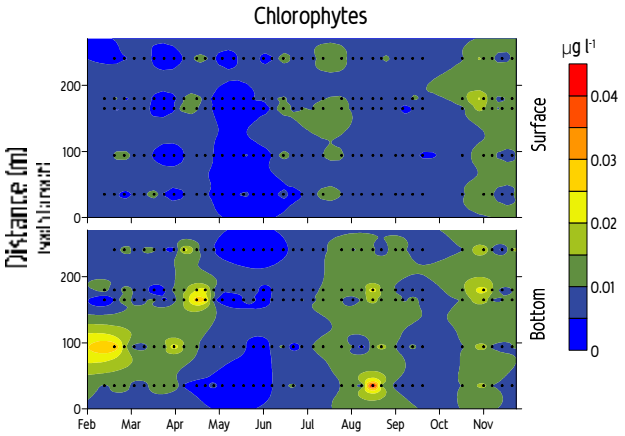

Figure III.A.7. Spatiotemporal contour plot for chlorophytes.

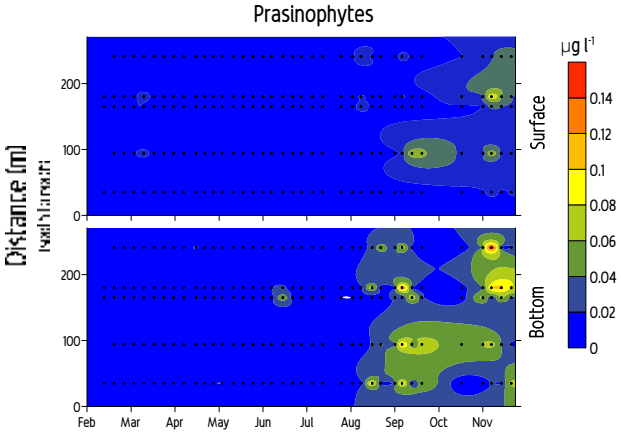

Figure III.A.8. Spatiotemporal contour plot for prasinophytes.

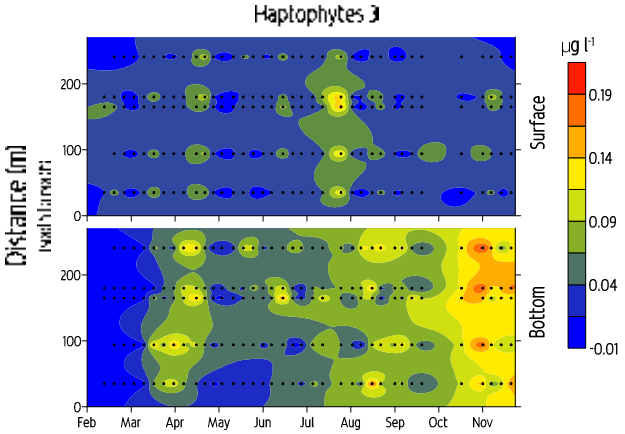

Figure III.A.9. Spatiotemporal contour plot for haptophytes group 3 (mostly coccolithophores).

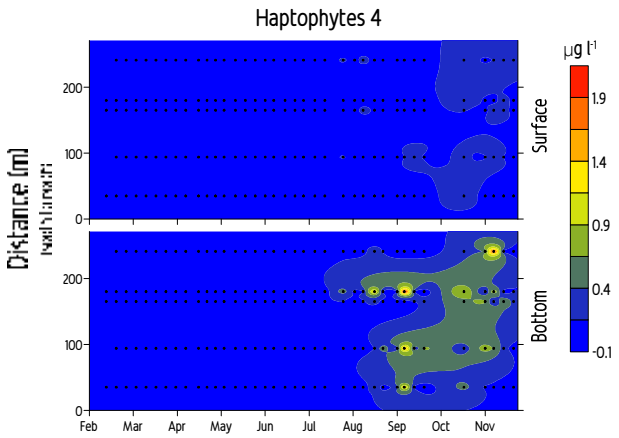

Figure III.A.10. Spatiotemporal contour plot for haptophytes group 4 (mostly prymnesiophytes).

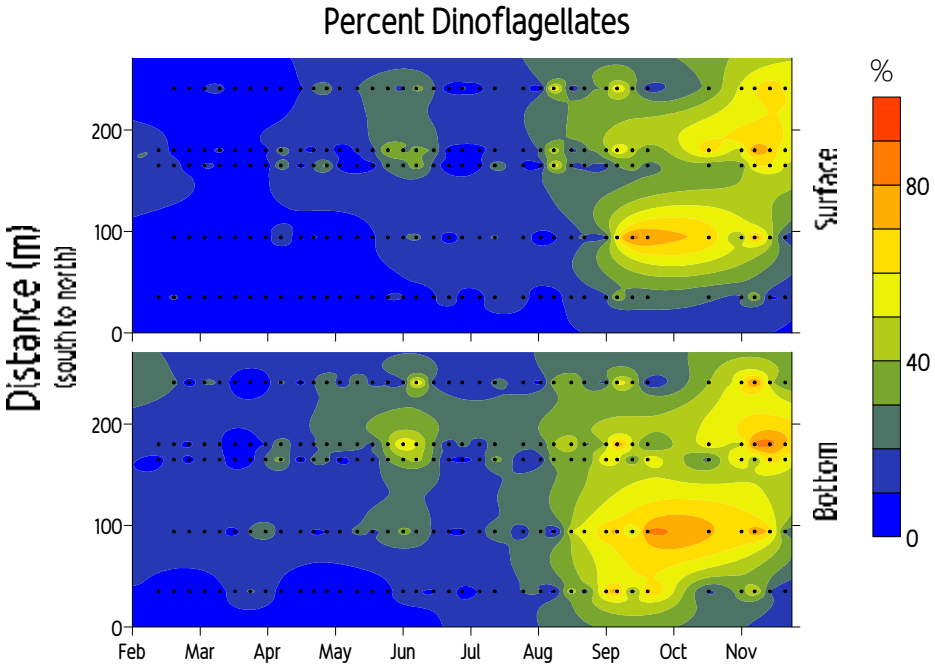

Figure III.A.11. Biomass contribution of dinoflagellates to the total phytoplankton community, expressed as a percentage relative to chlorophyll *a*.

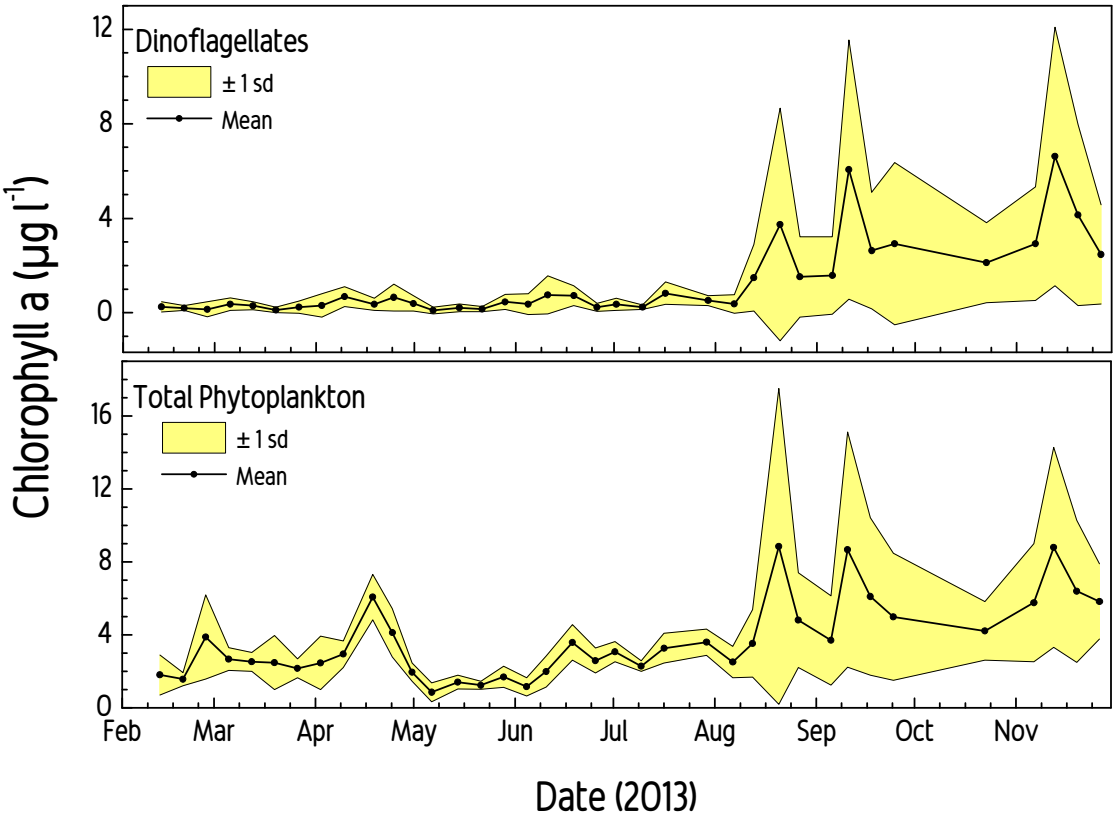

Figure III.A.12. Mean concentrations of total phytoplankton and dinoflagellates in Mangrove Lagoon over the study period. The colored band indicates ± 1 standard deviation.

The relationship between daily rainfall and the concentrations of the total phytoplankton community and dinoflagellates was examined by overlaying the data from the weekly sampling (fig. III.A.13). From February to June, there was no obvious correlation between rainfall and concentrations of total phytoplankton or dinoflagellates. Three major blooms of phytoplankton and dinoflagellates in August, September, and November followed heavy rain events. Since these rain events were not correlated with large increases in nutrient concentrations (see Fig. II.5), the data suggest that either reductions in salinity, or mixing associated with storms (wind and rainfall), stimulated the algal blooms. Another possibility is that nutrient inputs, via runoff or atmospheric deposition (nutrients and particulates in dust and rain), were rapidly assimilated by phytoplankton such that ambient nutrient concentrations were low at the time of sampling. Comparisons of dinoflagellate and total phytoplankton concentrations with nutrient (N and P) concentrations suggest a single bloom of phytoplankton (mostly diatoms) occurred following elevated nutrient levels in April (Fig. II.A.14). Other blooms were not directly associated with higher than normal nutrient concentrations.

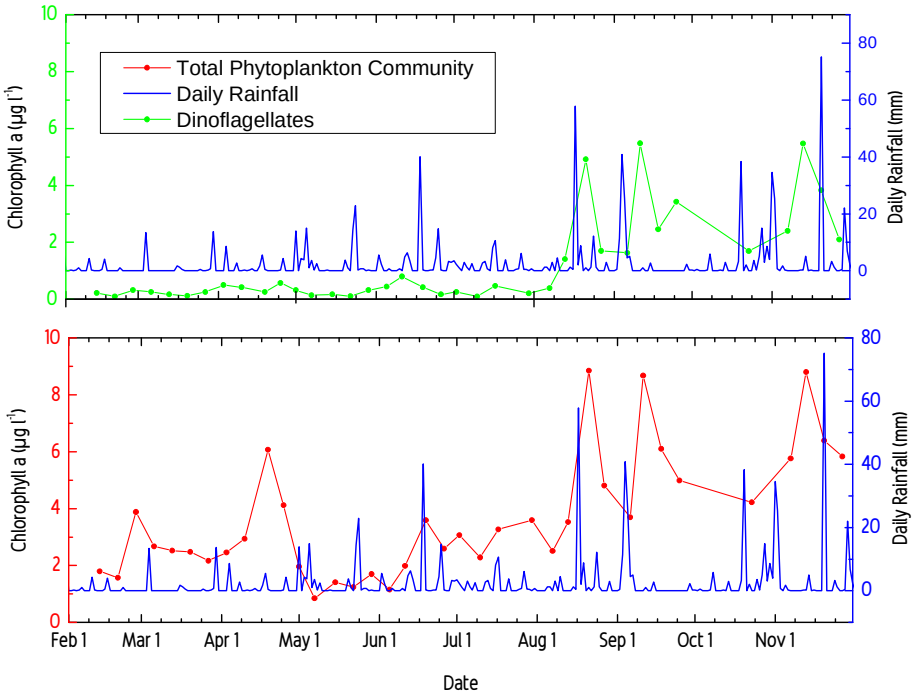

Figure III.A.13. Comparison of total phytoplankton community and dinoflagellate concentrations with daily rainfall in Mangrove Lagoon.

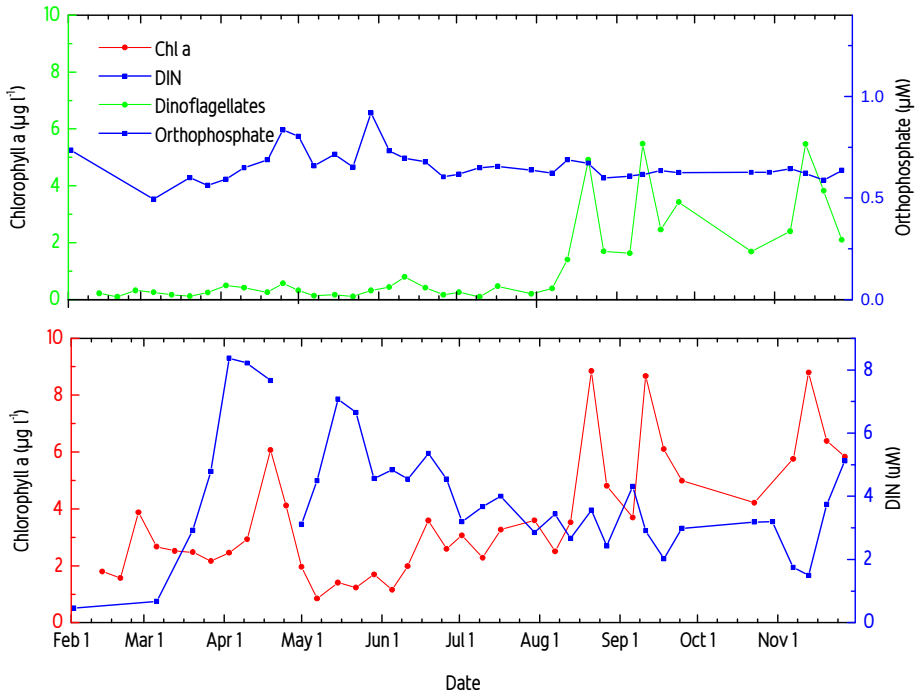

Figure III.A.14. Comparison of total phytoplankton community and dinoflagellate concentrations with concentrations of the nutrients total dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (orthophosphate) in Mangrove Lagoon.

SUMMARY

The phytoplankton community in Mangrove Lagoon is composed primarily of diatoms, dinoflagellates, cyanobacteria, and prymnesiophytes. The relative contributions of each of these groups seem to vary seasonally. The early summer diatom bloom was correlated with an increase in dissolved inorganic nitrogen (DIN) concentrations while the late summer/fall blooms were not related to DIN concentrations or N:P ratios. The fall blooms seemed to co-occur with rain events. During the day, phytoplankton concentrations are higher in the bottom waters, possibly due to negative phototaxis in the shallow lagoon waters. Phytoplankton concentrations were higher during the late summer and fall months, with dinoflagellates composing as much as 90% of total phytoplankton biomass. Both variability and total biomass of phytoplankton was highest in the fall months (August-November). Heavy rainfall events in the late summer and fall (August – November) seem to promote blooms of phytoplankton including dinoflagellates. In general, most rainfall events do not result in increased nutrient concentrations. However, it is possible that nutrient uptake occurred at time scales shorter than our weekly sample collections.

Collectively these results suggest a diverse phytoplankton community which experiences periodic blooms, as high as 28 μg l^{-1}, that are loosely correlated with rainfall events and elevated nutrient concentrations. The bloom species appear to be limited to either diatoms or dinoflagellates.

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(B) Dormant dinoflagellate cysts in sediments of Mangrove Lagoon (Lane & Reidhaar)

METHODS

Field work in Mangrove Lagoon and Salt River Bay was conducted from May 22 to May 26, 2012. During the first expedition we collected 55 surface sediment samples (50 from Mangrove Lagoon, three from Salt River Bay near the mouth of Mangrove Lagoon, and one sample from Sugar Bay and Triton Bay, respectively). Surface sediment samples from Mangrove Lagoon were collected at ~25 m intervals using an Ekman-style dredge deployed from a Boston Whaler (Fig. III.B.1). We also collected surface water samples for nutrient analysis from every other sediment sampling position using a using a 50 ml syringe fitted with a 0.3 micron glass fiber filter in order to remove particulates (Fig. III.B.1). Samples for nutrient analysis were spiked with mercuric chloride and stored in 20 ml I-CHEM vials until processing. All Sample positions were established and recorded using a handheld GPS receiver. We also used dredge depths to establish a detailed bathymetry for Mangrove Lagoon (Fig. III.B.2).

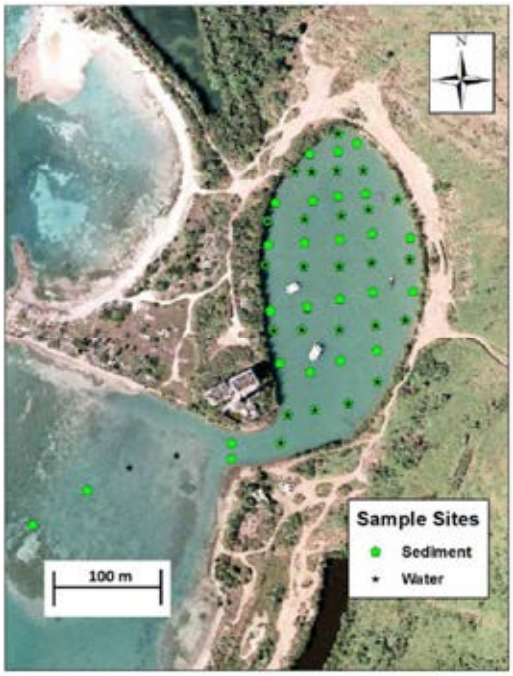


Figure III.B.1. Surface sediment and water sampling locations in and near Mangrove Lagoon

A second expedition to Mangrove Lagoon and Salt River Bay was conducted from May 15 to May 22, 2013. During this expedition, six short sediment cores were collected from a Boston Whaler or a floating platform as push cores through use of a PVC pipe fitted with a piston at locations where surface sediment contained a high concentration of cysts (as indicated by surface sediment sample analysis on samples from the first expedition). Each core recovery location was identified and recorded using a handheld GPS receiver. In total, six cores were recovered from the lagoon to ensure that temporal variations in different locations could be compared (Fig. III.B.3). At each coring location sediments were collected to the point of refusal. Every coring location was underlain by very coarse gravel or bedrock that was impenetrable using our coring equipment.

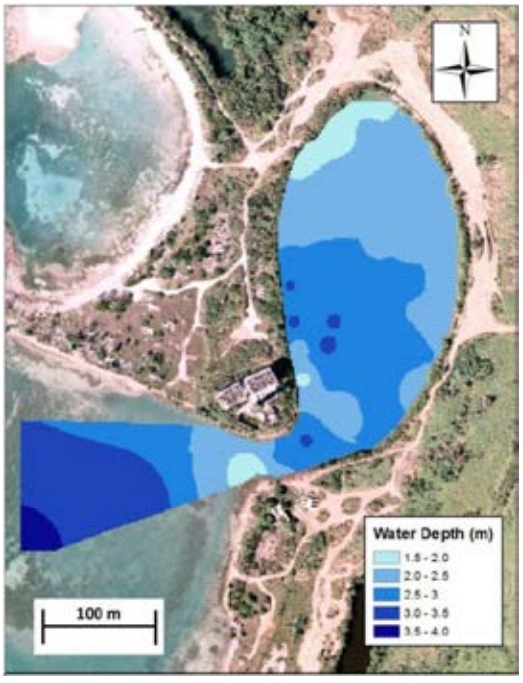


Figure III.B.2. Bathymetry of Mangrove Lagoon based on sediment dredge depths

Short-Lived Radioisotope Chronologies A total of 78 sediment subsamples from each coring location were submitted to Dr. Claudia Benitez-Nelson at the University of South Carolina for Pb-210 and Cs-137 short-lived radioisotope analyses to establish chronologies for the cores and sedimentation rates in Mangrove Lagoon. Sub-samples from each sediment core were taken at 1–4 cm intervals, dried at 50°C, ground to a fine powder using a mortar and pestle, and then sealed with two-part epoxy in snap-cap vials and shipped to the University of South Carolina for analysis.

Bulk Sediment Geochemistry Bulk density, combustible organic matter content, water content, and carbonate content were determined for surface sediment and core sub-samples by measuring mass loss on ignition (LOI) at 100°C, 550°C, and 1000°C, respectively (Dean, 1974). Percent organic carbon was obtained using a Costech Elemental Analyzer paired with a Thermo Delta V continuous flow mass spectrometer. For carbon isotope analysis, 10% HCl was added to the sediment to remove carbonates. Samples were then dried on a hot plate at 85 °C and ground to a fine powder using a mortar and pestle to homogenize the material. Dried sediment was then loaded into tin capsules for isotope analysis. For nitrogen isotope analysis untreated sediment samples were dried at 50 °C overnight so they could be ground to a fine powder and homogenized before loading sediment into tin capsules. Isotope analyses for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were conducted using a Costech Elemental Analyzer paired with a Thermo Delta V continuous flow mass spectrometer. Isotopic compositions were reported in δ-per mill notation based on the Vienna-Pee Dee belemnite standard for marine-carbonate using the following equation:

$$\delta X \text{ (per mil)} = 1000\ [(R_{sample}/R_{standard}) - 1],$$

Where X = ^{13}C or ^{15}N; and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively

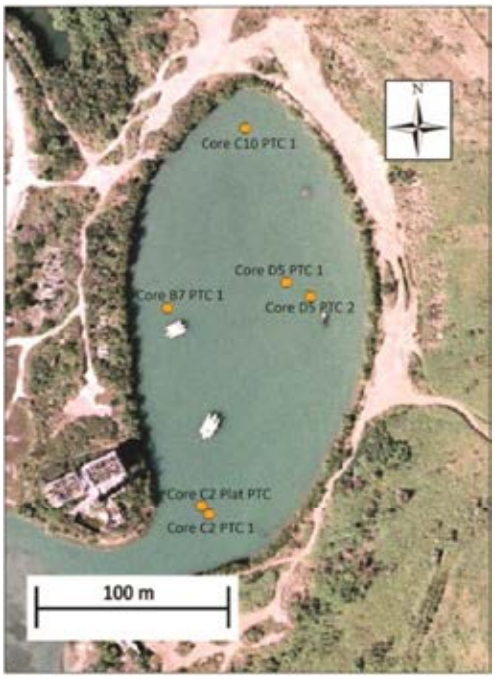


Figure III.B.3. Sediment core locations in Mangrove Lagoon

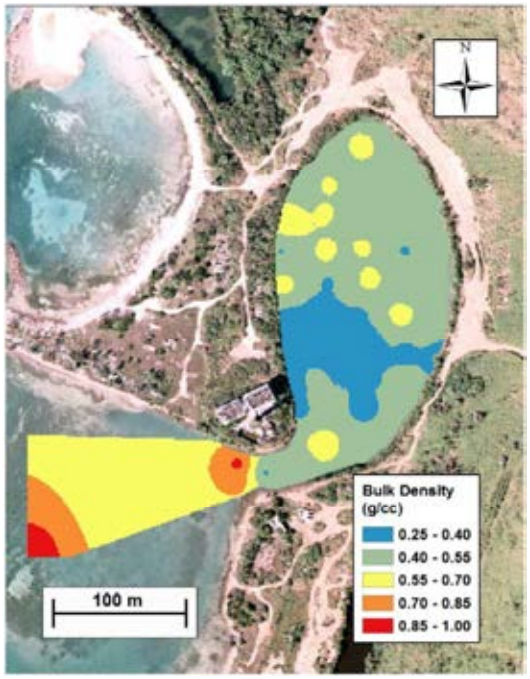


Figure III.B.4. Bulk density (g/cc) of surface sediments collected from Mangrove Lagoon.

Dinoflagellate Cyst Isolation and Quantification Dinoflagellate cysts from surface sediments and core subsamples were isolated using modified pollen processing techniques (Faegri and Iverson, 1989). Bulk sediment samples of 2.5 ml were sieved using nested 125 and 45 µm sieves, keeping only material captured by the 45 µm sieve as *Pyriodinium bahamense* cysts are typically larger than 45 µm and smaller than 125 µm (Williams and Bujak, 1985). A single *Lycopodium* tablet with a known spore count was added to each sample to allow for cyst quantification based on the known number of spores in the marker. Eight ml of 10% HCl was added to each sub-sample to break down calcium carbonate and 8 ml of HF was added to break down silicate materials. The sub-samples were rinsed with hot deionized water, centrifuged for three minutes at 2500 rpm, and decanted between treatments. Once all unwanted materials were broken down and removed, safranin staining dye was added to the sample. Each sample was then mounted in silicone oil on slides and *P. bahamense* cysts were counted at 400X magnification. Cysts were counted up to a minimum of 300 control spores. Cysts were visually identified based on the descriptions of Matsuoka and Fukuyo (2000). Cyst concentrations are presented as *P. bahamense* cysts g dry sediment^{-1}.

RESULTS

Short-Lived Radioisotope Chronologies Due to the considerable analysis time required for Pb-210 and Cs-137 activity measurements, these analyses are still underway at the University of South Carolina. Preliminary results from the C2 Plat PTC core indicate that the sediments are in stratigraphic order and that the basal sediments have Pb-210 activities that are below detection. Based on the half-life of Pb-210 (22.5 years) and the sensitivity of the instrumentation, this means that sediments as old as 100 years were likely collected from this coring position. Preliminary results from Cs-137 analyses on the C2 Plat PTC core indicate maximum activities near 22 cm depth, meaning this depth likely corresponds to the year 1963 (Robbins et al., 2000; Ryan-Mishkin et al., 2009). Preliminary Pb-210 decay profiles indicate average sedimentation rates of ~0.72 cm/yr over the last two to three decades (Fig. III.B.4A). A sharp change in the Pb-210 decay profile at ~30 cm may indicate a hiatus in sedimentation or removal of sediments or deep sediment mixing during resort construction. Preliminary Pb-210 results from the D5 coring position (cores D5 PTC1 and D5 PTC2) indicate substantial mixing of the uppermost sediments most likely due to physical mixing (waves or currents) and bioturbation, which makes it difficult to establish a sedimentation rate at this position.

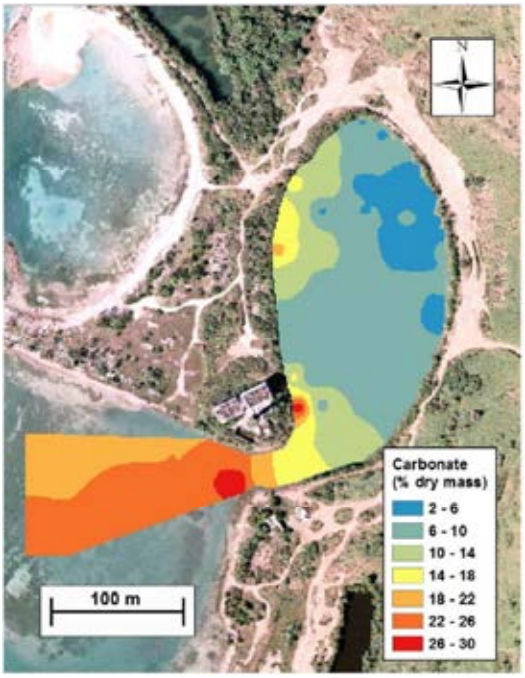


Figure III.B.5. Carbonate content (% dry mass) of surface sediments collected from Mangrove Lagoon.

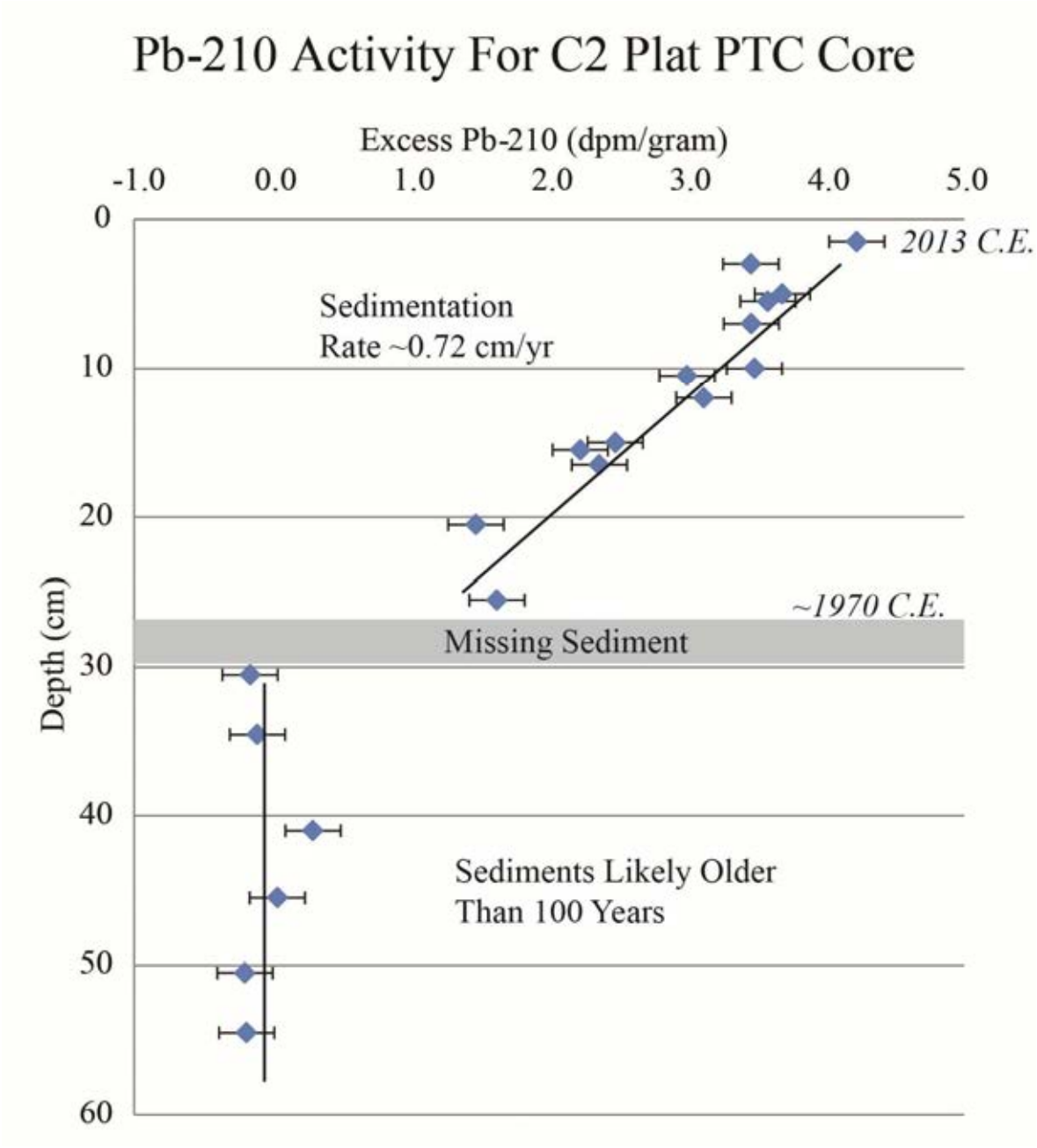


Figure III.B.4A. Excess 210 Pb activity in core C2 Plat PTC

Bulk Sediment Geochemistry Bulk density values for Mangrove Lagoon surface sediment samples varied from 0.25–1.0 g cm^{-3} (Fig. III.B.4). Surface sediment carbonate percentages varied from 2–30% dry mass with higher values near the mouth of the lagoon (Fig. III.B.5). Organic carbon contents are highest near the center and eastern shoreline of the lagoon varying from 2–7% dry mass (Fig. III.B.6). Organic matter percentages within surface sediment samples range from 7–19% dry mass and are generally highest along the eastern shoreline (Fig. III.B.7). Bulk density within all sediment cores ranged from 0.23–2.39 g cm^{-3} with organic matter percentages from 2.36–32.16% dry mass. The sediment core samples have organic carbon contents between 0.944 and 12.86% dry mass and carbonate percentages of 0.17–97.66% dry mass (Figs. III.B.8–III.B.11).

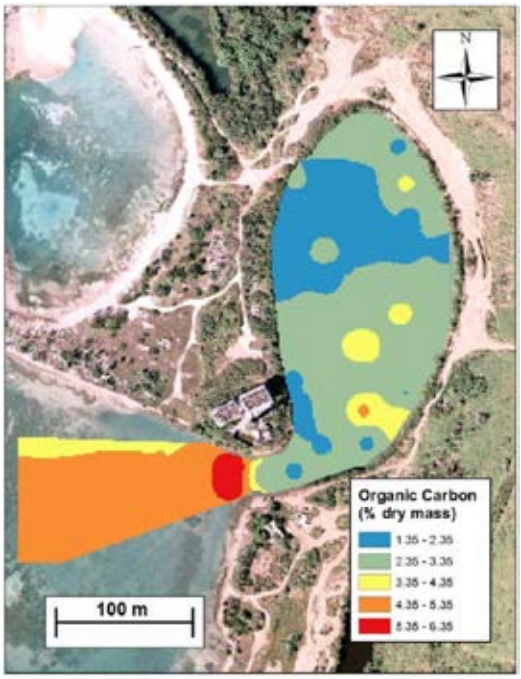


Figure III.B.6. Organic carbon content (% dry mass) of surface sediments collected from Mangrove Lagoon.

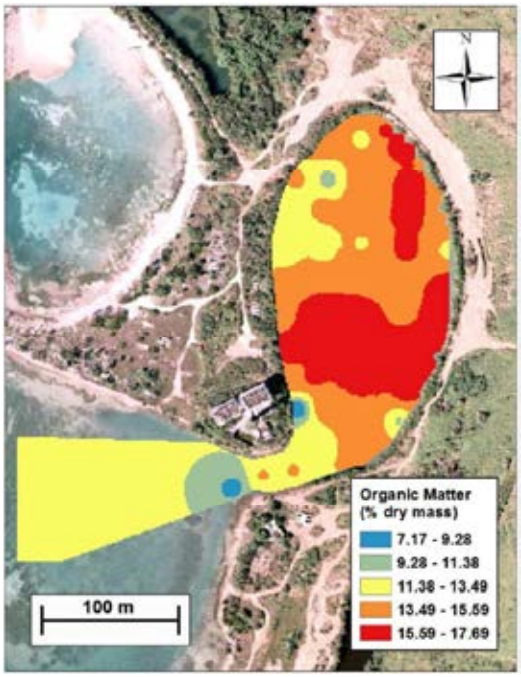


Figure III.B.7. Combustible organic matter content (% dry mass) of surface sediments from Mangrove Lagoon

Sedimentary $\delta^{15}N$ values varied from 2.1‰ to 4.0‰ in Mangrove Lagoon surface sediments. Sediments with relatively high $\delta^{15}N$ values are located near the western shore of the lagoon (Fig. III.B.12). Sedimentary $\delta^{15}N$ values within all six sediment cores ranged from 1.7‰ to 6.5‰ (Fig. III.B.13). The core recovered near the mouth of the lagoon demonstrates a pattern of decreasing $\delta^{15}N$ values with depth, while the cores from the western and northern region of the lagoon demonstrate increasing $\delta^{15}N$ values with depth. Samples taken from the adjoining bays (Sugar and Triton Bay) were found to have $\delta^{15}N$ values of 3.7‰ and 2.1‰, respectively. Two surface

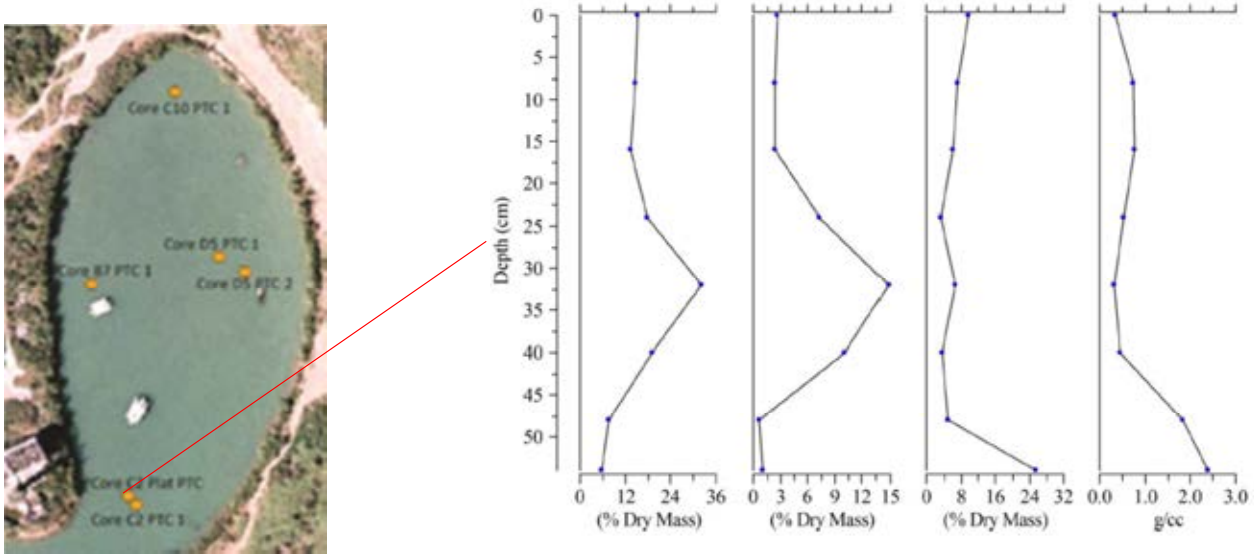


Figure III.B.8. Combustible organic matter content, organic carbon content, carbonate content, and bulk density of sediments in core C2 Plat PTC

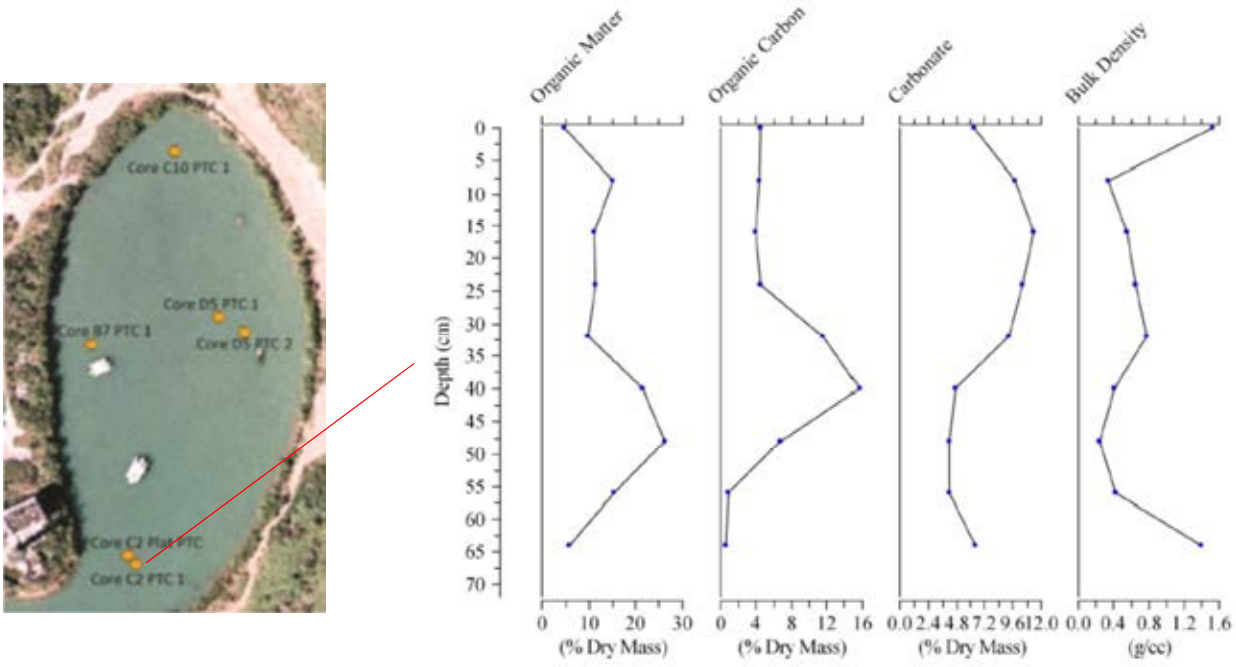


Figure III.B.9. Combustible organic matter content, organic carbon content, carbonate content, and bulk density of sediments in core C2 PTC1

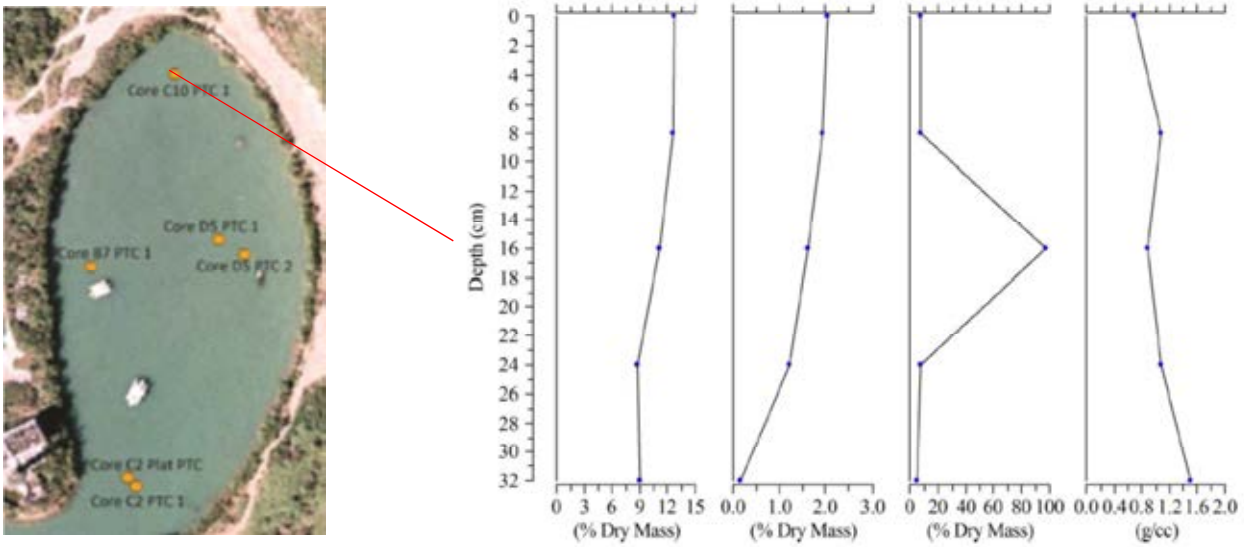


Figure III.B.10. Combustible organic matter content, organic carbon content, carbonate content, and bulk density of sediments in core C10 PTC1

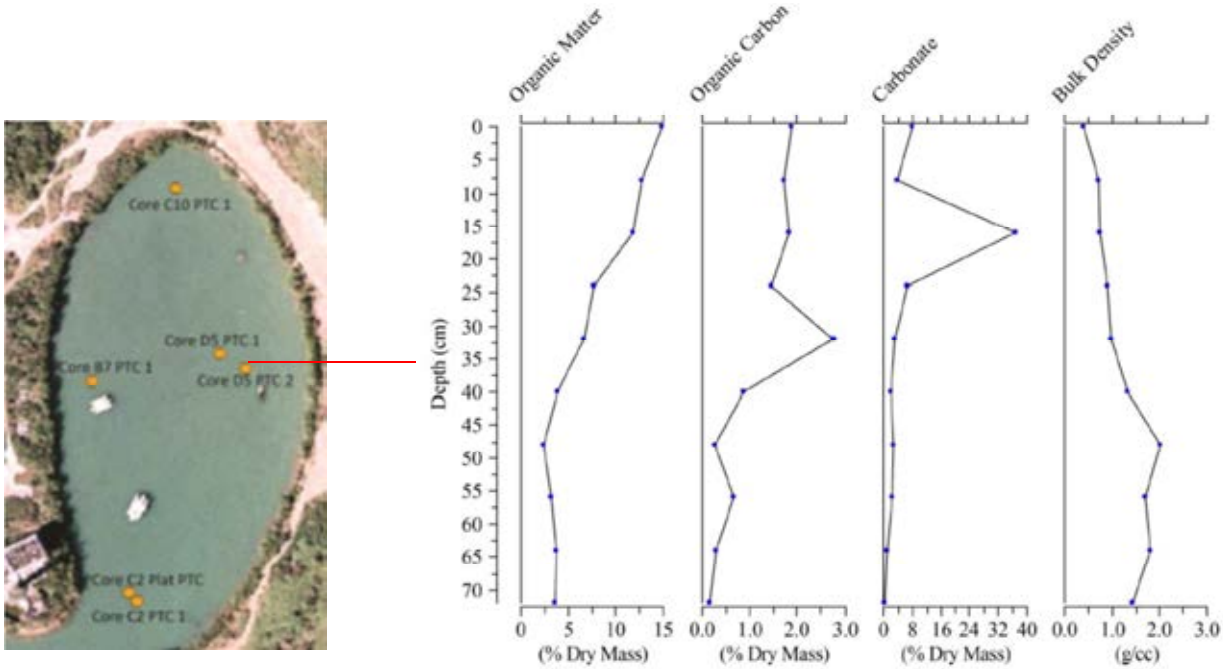


Figure III.B.11. Combustible organic matter content, organic carbon content, carbonate content, and bulk density of sediments in core D5 PTC2.

sediment samples recovered from Salt River Bay have $\delta^{15}N$ values of 2.5‰ and 2.7‰, respectively. Generally, more positive $\delta^{15}N$ values are indicative of greater primary productivity or decreased nitrogen availability (Brenner et al., 1999).

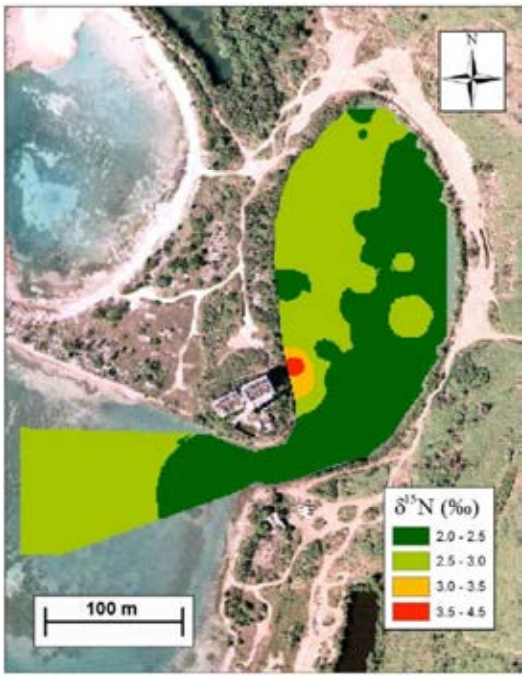
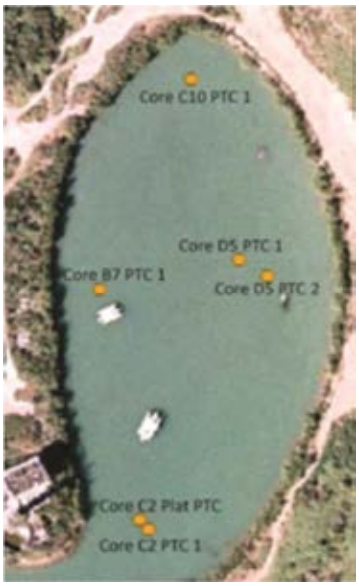


Figure II.B.12. Stable nitrogen isotope composition ($\delta^{15}N$) of surface sediments collected from Mangrove Lagoon.

Surface sediment $\delta^{13}C$ values range from -25.1‰ to -19.2‰ with higher values near the mouth of the lagoon and in Salt River Bay and lower values toward the north end of the lagoon (Fig. III.B.14). Carbon isotope values from Sugar and Triton Bay were -25.5‰ and -25.3‰, respectively. Two surface sediment samples recovered from Salt River Bay had $\delta^{13}C$ values of -19.3‰ and -20‰. Sedimentary $\delta^{13}C$ values of deeper sediments recovered during coring range from -22‰ to -26‰ (Fig. III.B.15). The relatively negative $\delta^{13}C$ values of Mangrove Lagoon sediment samples indicate a significant contribution of terrestrial carbon to the sedimentary organic carbon pool, most likely originating from fringing mangroves (Lane et al., 2013).

Surface sediment C:N ratios ranged from 4 to 21 with higher values occurring along the eastern side of the lagoon (Fig. III.B.16). Elemental C:N ratios greater than 20 are



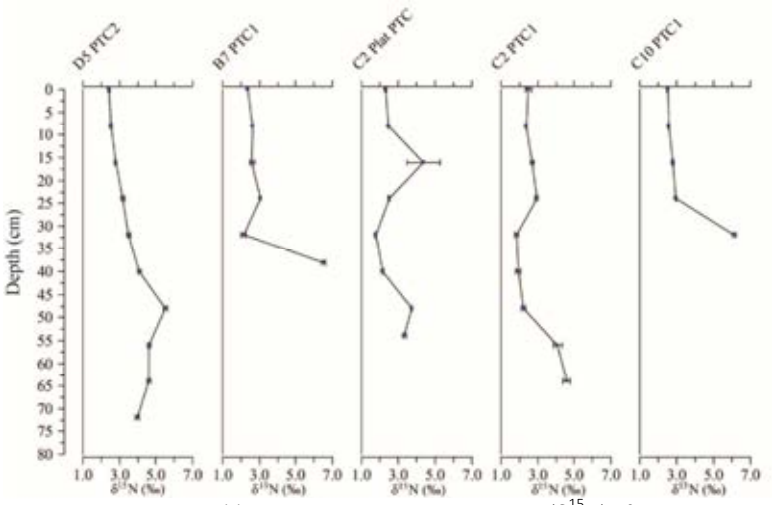


Figure II.B.13. Stable nitrogen isotope composition ($\delta^{15}N$) of core sediments collected in Mangrove Lagoon.

attributable to organic matter originating from terrestrial plants while values less than 10 are typical of organic matter from aquatic organisms. Elemental C:N values between 10 and 20 are indicative of a mixed terrestrial-

aquatic organic matter pool (Talbot and Johannessen, 1992). The elevated C:N ratios along the eastern shoreline indicates greater terrestrial matter influx from the uplands to the east, but the values throughout the lagoon are indicative of a well-mixed aquatic and terrestrial organic matter source. Ratios within sediment cores generally increase with depth indicating greater terrestrial organic matter influxes prior to the 1970's based on preliminary Pb-210 chronologies (Fig. III.B.18). Core C10 PTC1 taken from the northern end of the lagoon has a relatively low C:N ratio throughout the core. The C:N ratios for Sugar and Triton Bay were 13 and 27 respectively. Carbon to nitrogen ratios for two surface samples recovered from Salt River Bay were 27 and 20.

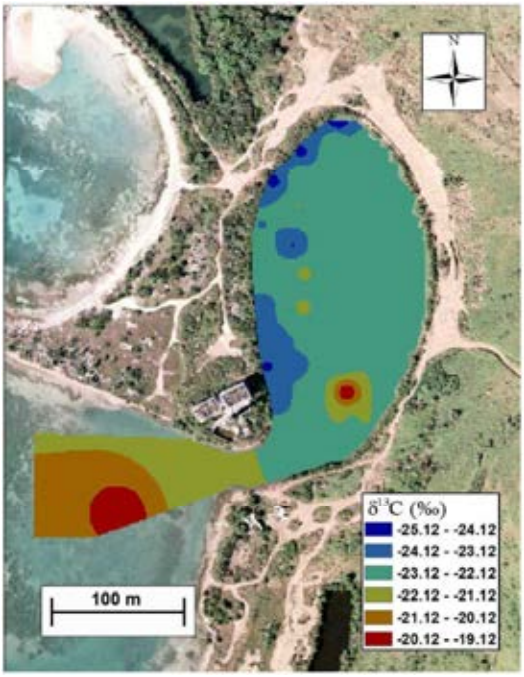


Figure III.B.14. Stable carbon isotope composition ($\delta^{13}C$) of surface sediments collected from Mangrove Lagoon.

Spatial and Temporal Distribution of *Pyrodinium bahamense* cysts in Mangrove Lagoon Sediments *Pyrodinium bahamense* cyst concentrations in surface sediments of Mangrove Lagoon range from 0–466 cysts g dry sediment^{-1} with higher concentrations in the northern and eastern quadrants of the lagoon (Fig. III.B.18). Surface samples from Salt River Bay, Triton Bay, and Sugar Bay had very low *P. bahamense* cyst concentrations that never exceed 38 cysts g dry sediment^{-1}. Concentrations of *P. bahamense* cysts in Mangrove Lagoon indicate relatively low dinoflagellate abundance compared to other bioluminescent lagoons in the region. Puerto Mosquito, Vieques, Puerto Rico has an average cyst concentration of 4697 cysts g of wet sediment^{-1} (Hereid, 2007). Laguna Grande, Puerto Rico has cyst concentrations near 4776 cysts g of wet sediment^{-1} (Lane et al., 2013). The maximum cyst concentration in surface

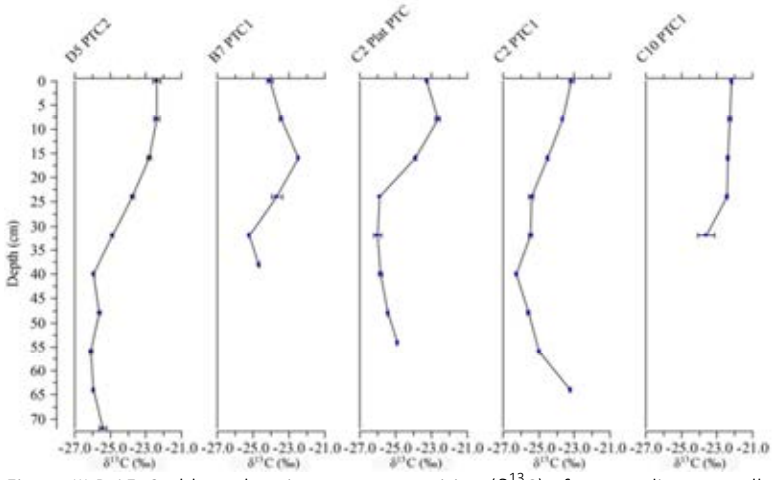


Figure III.B.15. Stable carbon isotope composition ($\delta^{13}C$) of core sediments collected from Mangrove Lagoon.

sediments of Mangrove Lagoon is 255 cysts g of wet sediment^{-1} from a surface sample collected on the eastern side of the lagoon (Fig. III.B.19).

Sediment core collections in Mangrove Lagoon

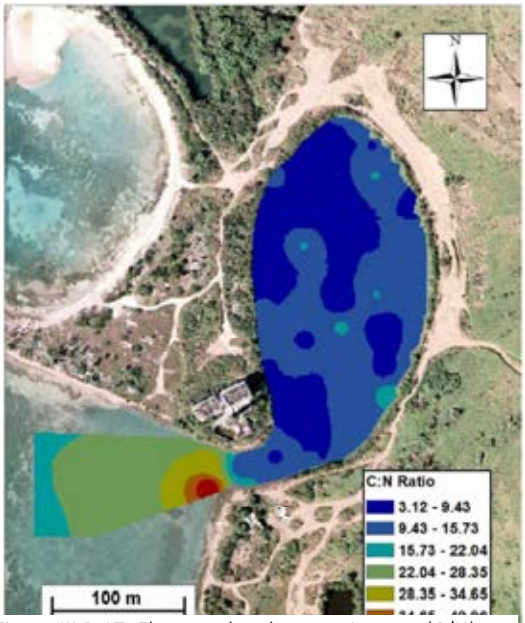


Figure III.B.17. Elemental carbon to nitrogen (C/N) ratios of surface sediments collected from Mangrove Lagoon.

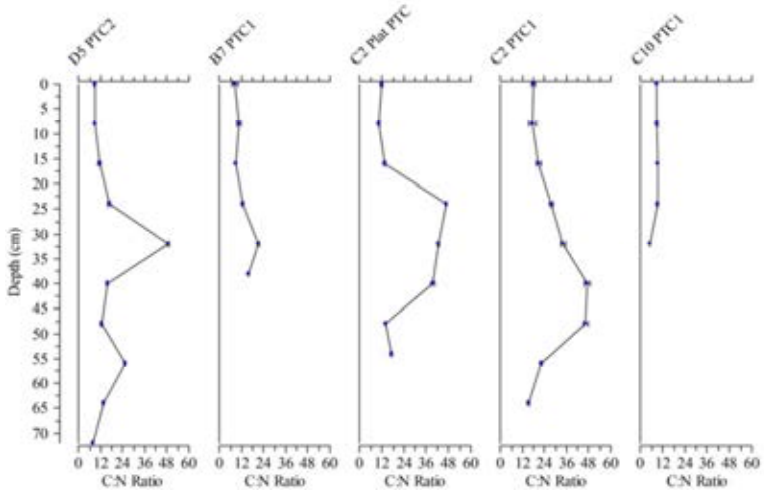


Figure III.B.17. Elemental carbon to nitrogen ratios (C/N) of core sediments collected from Mangrove Lagoon.

Maximum cyst concentrations (~1000 cysts g dry sediment^{-1}; Fig. 23) were observed at depths of ~26 cm in the sediment core collected from the C2 PTC 1 coring location (Fig. III.B.3) in Mangrove Lagoon. While short-lived isotope analyses are still underway, preliminary Pb-210 analyses indicate sedimentation rates approaching 0.72 cm yr^{-1} at the adjacent C2 Plat PTC coring location (Fig. III.B.3). This would mean that cyst concentrations peaked approximately 20 years ago at this core location. Cyst concentrations in the other cores collected from the lagoon indicate a similar pattern of cyst sedimentation with maximum cyst concentrations occurring at depths of 10–30 cm, and declining to the present (Fig.I II.B.20).

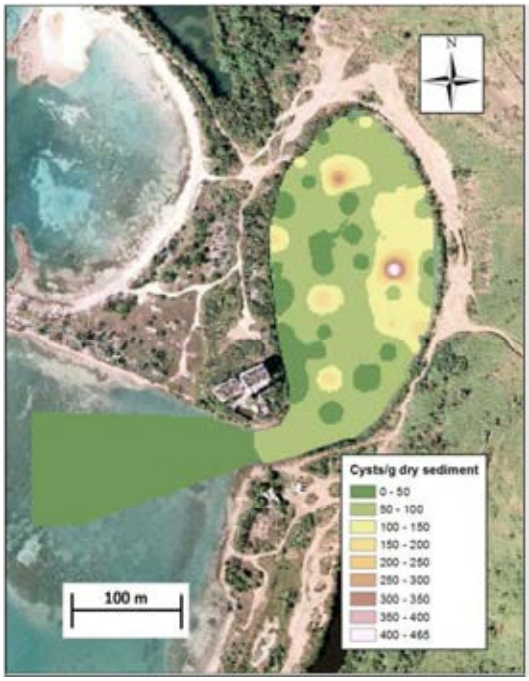
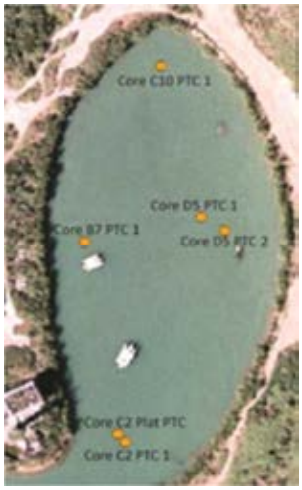


Figure III.B.18. *Pyrodinium bahamense* cyst concentrations in surface sediments of Mangrove Lagoon.



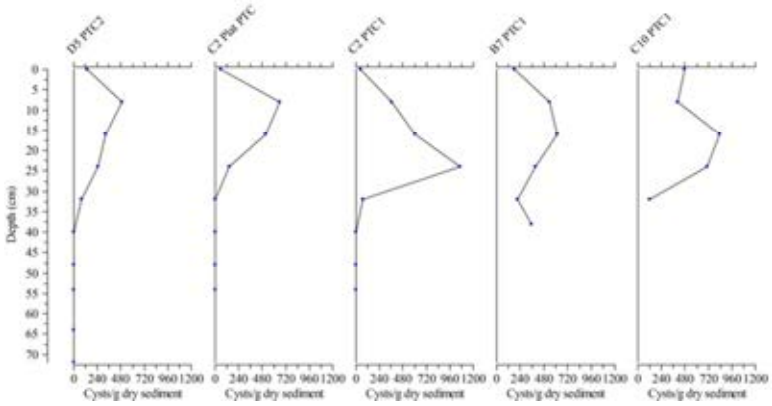


Figure III.B.19. *Pyrodinium bahamense* cyst concentrations in sediment cores collected from Mangrove Lagoon.

CONCLUSIONS

Spatial heterogeneity of *Pyrodinium bahamense* cyst concentrations in Mangrove Lagoon sediments indicate preferential habitats for this dinoflagellate along the northern and eastern shorelines. Informal interviews with bioluminescence tour operators in Salt River Bay indicated that the brightest bioluminescence typically occurs along the northern and eastern shorelines of Mangrove Lagoon. Comparison between our nutrient concentration and sediment geochemistry data do not indicate any clear explanations as to why *P. bahamense* prefers the

northeastern portion of the lagoon. However, the noticeably lower $\delta^{15}N$ value of surface sediments along the eastern half of the lagoon may provide a hint that nutrient availability is a limiting factor for dinoflagellate populations.

Generally speaking, more negative $\delta^{15}N$ values are indicative of greater nitrogen availability in aquatic environments. The morphology of Mangrove Lagoon combined with persistent northeasterly trade winds likely forces water to circulate counter-clockwise from the opening to Salt River Bay along the southwestern shoreline (Fig. III.B.20). As seawater enters Mangrove Lagoon, phytoplankton seem to be quickly utilizing available N, particularly the preferred ^{14}N, leaving the water enriched in ^{15}N by the time it reaches the western shoreline. This interpretation is supported by the notable increase in $\delta^{15}N$ values along the western side of the lagoon compared to the eastern side of the lagoon. The relatively high sedimentary organic matter content and dissolved nitrate concentrations in the eastern half of the lagoon compared to the western half of the lagoon also indicate greater primary productivity and support this interpretation. While nutrient assays showed no significant effect of nitrogen or phosphorous additions to *P. bahamense* growth, it is still reasonable to assume that nutrient availability is playing an important role in *P. bahamense* distributions in Mangrove Lagoon as *P. bahamense* may be acquiring nutrients at the sediment-water interface during diurnal vertical migrations. Other phytoplankton (diatoms, cyanobacteria, etc.) are likely utilizing these nutrients in the water column and then contributing to the sedimentary nitrogen and phosphorous pools after deposition. Thus, water column and sedimentary nutrient availability could be an important variable dictating *P. bahamense* distributions in Mangrove Lagoon.

Temporal variations in *Pyrodinium bahamense* cyst concentrations indicate that *P. bahamense* has not always been abundant in Mangrove Lagoon and that *P. bahamense* populations may be on the decline. Based on the preliminary chronology, the appearance of *P. bahamense* fossil cysts in the P2 Plat PTC sediment core appears to occur ~25 years ago and follows an apparent unconformity in the sedimentary profile likely resulting from dredging activities in the 1960's. Underlying this unconformity are sediments with no measurable Pb-210 activity indicating they are likely greater than 100 years old and are likely remnant sediments from the mangrove covered mudflat or salt pond that occupied the site prior to dredging activity. These lowermost sediments contained no *P. bahamense* cysts, leading us to conclude that colonization of Mangrove Lagoon by *P. bahamense* did not occur until the lagoon was connected to Salt River Bay. Relatively high *P. bahamense* cyst concentrations up to a depth of 24 cm in all sediment cores indicate that bioluminescence has been present for multiple decades in Mangrove Lagoon subsequent to dredging. However, cyst concentrations decline in the surface sediments of all sediment cores, potentially indicating a fairly recent decline in *P. bahamense* populations. An alternate explanation for this decline could be that the dinoflagellates are not forming cysts as frequently as they have in the past, but this hypothesis is a difficult one to test without long-term monitoring. This pattern of recent declines in cyst abundance is in contrast to the findings of Lane et al. (2013) who documented maximum concentrations of *P. bahamense* cysts in the surface

sediments of bioluminescent Laguna Grande, Puerto Rico relative to older sediments of the lagoon. Thus, based on the available data and contrast with Laguna Grande, we favor the more conservative interpretation at this time that dinoflagellate populations may be declining in Mangrove Lagoon.

The mechanism responsible for such a decline is not clear from the available data, but one potential mechanism might be the formation of a longshore bar across the mouth of Mangrove Lagoon, which might be limiting water exchange between Mangrove Lagoon and Salt River Bay.

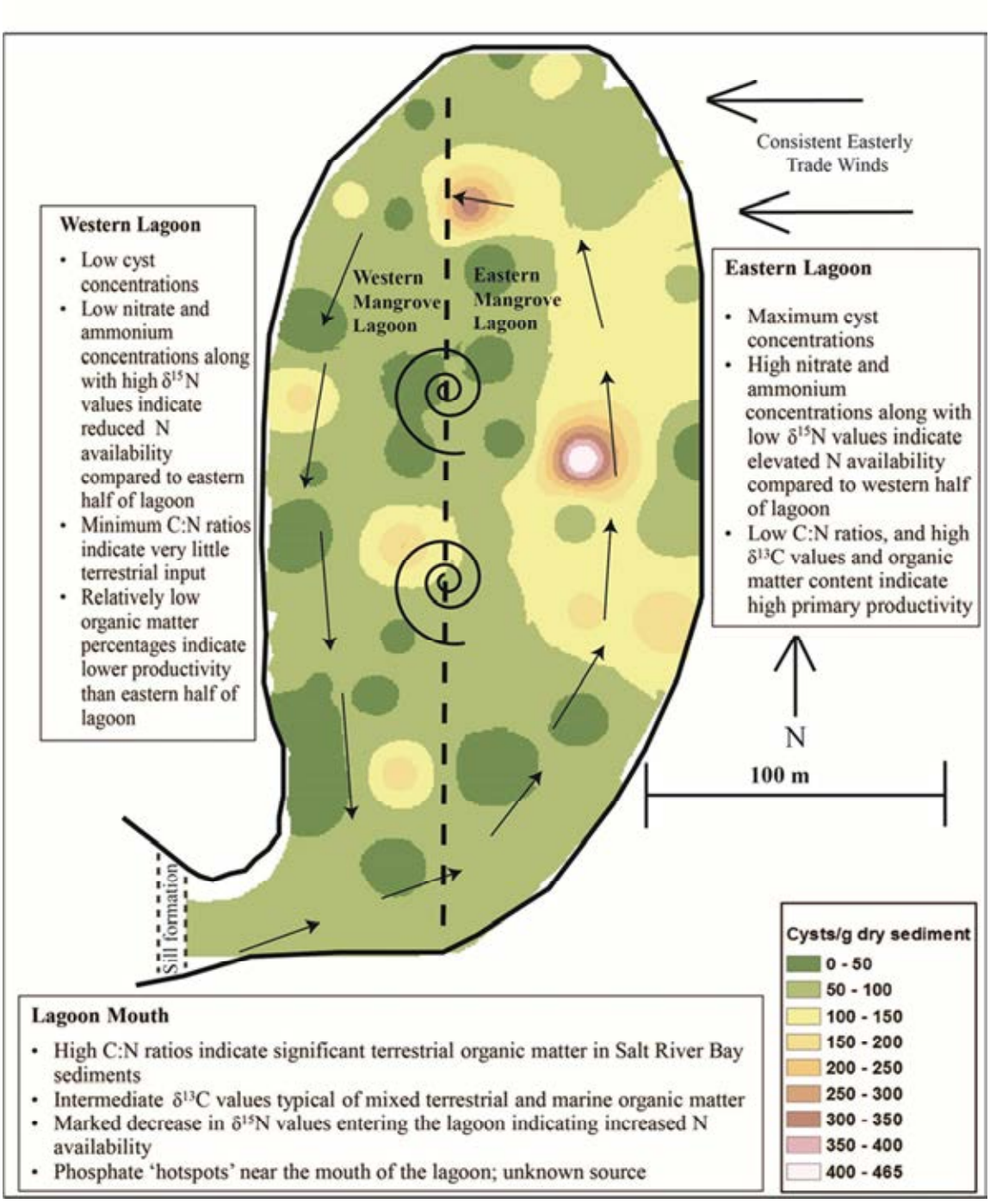


Figure III.B.20. Conceptual model of Mangrove Lagoon water circulation overlaying a map of surface sediment *Pyrodinium bahamense* cyst concentrations. Text boxes summarize relevant and potential processes affecting primary productivity, sediment geochemistry, and water chemistry in Mangrove Lagoon.

An open connection between Mangrove Lagoon and Salt River Bay along with a fringing mangrove community is likely important for sustaining high *P. bahamense* populations. Seliger et al. (1971) proposed three site criteria for the establishment of a bioluminescent bay or lagoon populated by *Pyrodinum bahamense* that include: (1) a shallow basin with a confined shallow entrance from the sea; (2) prevailing and daily-occurring winds blowing perpendicular to the entrance or into the bay; (3) mangrove drainage area surrounding the bay. Since that time, additional studies have also determined that *P. bahamense* flourish in bays or lagoons that also have low turbidity, are locations with relatively long water-residence times, have intermediate nutrient loads (<600 μg l^{-1} total nitrogen; <330 μg l^{-1} total phosphorous), and receive significant contributions of terrestrial, typically mangrove, carbon (Azanza et al., 2004; Florentine, 2007; Phlips et al., 2006). Based on available data, Mangrove Lagoon currently meets all of the proposed criteria. During bathymetric surveys, we documented an extremely shallow (<1.5 m deep) longshore bar forming across the mouth of Mangrove Lagoon. Continued sedimentation along this bar would eventually cutoff Mangrove Lagoon from Salt River Bay converting the lagoon into a fully isolated coastal lagoon similar to the unnamed lagoon to the north.

Based on our conceptual model of current flows and nutrient dynamics in Mangrove Lagoon (Fig. III.B.20), the connection between Salt River Bay and Mangrove Lagoon seems particularly important to the success of *P. bahamense*. Our geochemical and nutrient data indicate that waters entering the mouth of the lagoon may be an important source of nitrogen and phosphorous to resident planktonic communities. Such a significant change in coastal morphology would certainly affect flow dynamics and nutrient delivery to Mangrove Lagoon. Thus, any such change in Mangrove Lagoon morphology will also likely affect ecological processes in the waterbody, including *P. bahamense* population dynamics.

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OBJECTIVE IV. Conduct *in situ* nutrient addition bioassays to determine limiting factors and potential effects of elevated nutrient concentrations on dinoflagellate abundance (Pinckney, Zimberlin, Greenfield)

METHODS

Nutrient addition and leaf addition *in situ* bioassays were performed in January and May 2013. The water used in the bioassays was taken from the northeastern corner of Mangrove Lagoon, an area that historically has shown the highest intensity of bioluminescence at night and dinoflagellate cyst concentrations in the sediments (Reidhaar 2013). Water from the upper 1 – 2 m of the water column was collected using an integrated water sampler and dispensed into acid-washed 1 l Nalgene clear polycarbonate flasks. For the January nutrient bioassays, nutrients were added to achieve final concentrations of 20 µM NO_3^- (N), 10 µM PO_4^{-3} (P), N + P (NP), and a control (no nutrients added). For the May bioassays, nutrient concentrations were doubled (i.e., 40 µM N and 20 µM P) to insure that the additions were above ambient nutrient concentrations. There were four replicates for each treatment. The bottles were returned to the surface waters of the lagoon and covered with neutral density screen to reduce irradiance by ~40%. The samples were incubated for 48 h under ambient conditions. After the experiment was complete, treatment bottles were transported in the dark and inside coolers to the laboratory (University of the Virgin Islands) for subsequent processing. A subsample (1 -3 ml) of each replicate was visually inspected for *P. bahamense* using light microscopy. Aliquots (50-250 ml) of the incubation water were filtered under a gentle vacuum (<50 KPa) through a glass fiber filter (0.7 µm pore size, 25 mm dia. Whatman GF/F), immediately frozen, and stored at -80°C. The filters and filtrate were subsequently shipped to the University of South Carolina laboratory (Columbia, SC, USA) in a Dewar flask cooled with liquid N_2.


Bioassay bottles with mangrove leaf additions

Water for the mangrove leaf addition bioassay was collected and dispensed into bottles as described above. Senescent orange and yellow leaves were collected from mangrove trees surrounding the lagoon and placed into bioassay bottles along with water from the lagoon. The treatments were as follows: 1) 3 yellow leaves (Y3); 2) 3 orange leaves (O3); 3) 6 yellow leaves (Y6); 4) 6 orange leaves (O6) and 5) control (C), nothing added. The color variation is indicative of nutrient levels, age, and leachate (Benner et al. 1990a,b). There were 3 replicates of each treatment, and the bioassay was terminated after 48 h. Samples were filtered and stored as described above.

Analytical Methods Phytoplankton photopigment concentrations were measured using high performance liquid chromatography (HPLC) (Pinckney et al. 2001). Filters were lyophilized for 18-24 hours at -50 °C then extracted by

adding 750 µl of 90% aqueous acetone solvent followed by storage for 12-20 hours at -20°C. Filtered extracts (250 µl) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing) and a polymeric (Vydac 201TP54, 0.46 × 25 cm, 5 µm packing) reverse-phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol : 20% 0.5 M ammonium acetate) and solvent B (80% methanol : 20% acetone) was used for the mobile phase (Pinckney et al. 1996). Absorption spectra and chromatograms (440 ± 4 nm) were obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma) was used as an internal standard. Pigment concentrations were further analyzed using ChemTax (v. 1.95) to determine the relative abundance of major phytoplankton groups (Pinckney et al. 2001, Higgins et al. 2011). The initial pigment ratio matrix used for this analysis was derived from Mackey et al. (1996). The convergence procedure outlined by Latasa (2007) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed values. The major phytoplankton groups used for ChemTax categories were based on qualitative microscopic examinations of water from the bioassays.

Dissolved organic carbon (DOC) was measured with a Shimadzu TOC-V analyzer with an attached ASI-V autosampler. Filtrate (0.7 µm filtered) was analyzed for orthophosphate, dissolved inorganic nitrogen as nitrite/nitrate, and ammonium using a Lachat Quick-Chem 8000 nutrient auto-analyzer according to standard methodology (Johnson & Petty 1983; Zimmerman & Keefe 1991; Grasshoff 1999).

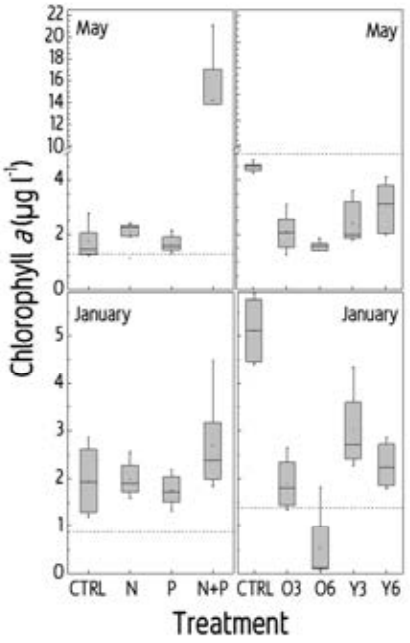

Figure IV.1. Boxplots of the nutrient and leaf bioassay results for total chlorophyll *a*. The horizontal dashed line indicates the initial concentrations (i.e., t_0)

RESULTS

For the January incubations, nitrate (NO_3^-) and orthophosphate (PO_4^{-3}) concentrations in the ambient seawater were 2.31 and 0.50 µM, respectively. For May, the concentrations were 1.10 and 0.50, respectively. The ambient N:P ratios for January and May were 5:1 and 2:1 and suggest N limitation. The nutrient additions of 20 – 40 µM NO_3^- and 10 – 20 µM PO_4^{-3} represented significant amendments above ambient concentrations. For the mangrove leaf bioassays, ambient DOC concentrations were 79 and 2,212 µM C for the January and May experiments, respectively. In January, each yellow leaf added an average of 222 µM C and each orange leaf added 204 µM C. For May, the yellow and orange leaves contributed 397 and 709 µM C $leaf^{-1}$, respectively.

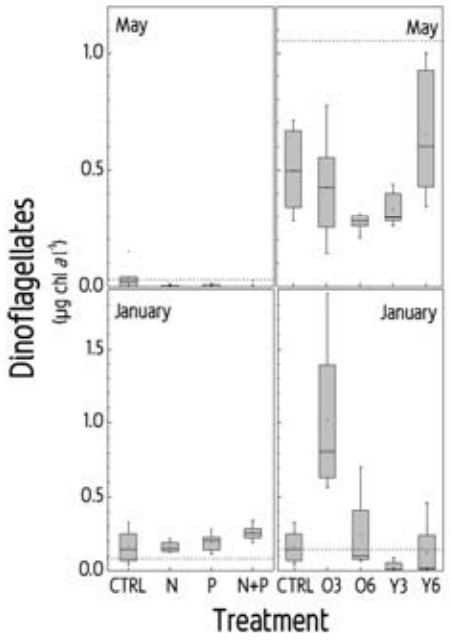

Figure IV.2. Boxplots of the nutrient and leaf bioassay results for dinoflagellates. The horizontal dashed line indicates the initial concentrations (i.e., t_0).

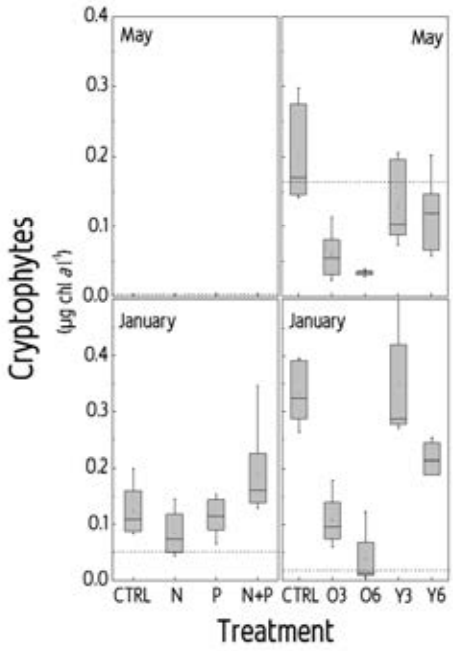

Figure IV.3. Boxplots of the nutrient and leaf bioassay results for diatoms. The horizontal dashed line indicates the initial concentrations (i.e., t_0).

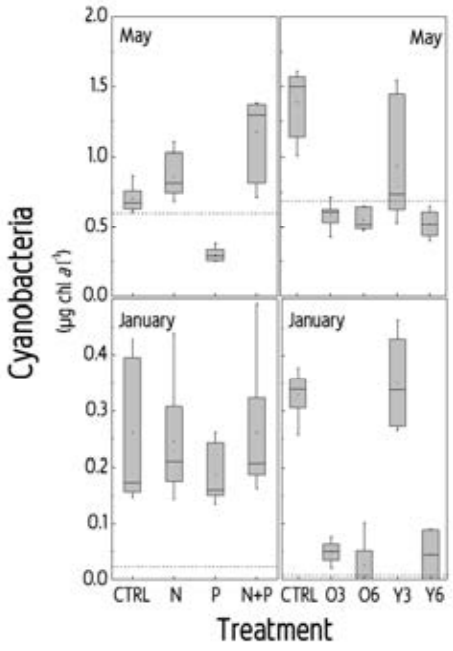

Figure IV.4. Boxplots of the nutrient and leaf bioassay results for cyanobacteria. The horizontal dashed line indicates the initial concentrations (i.e., t_0).

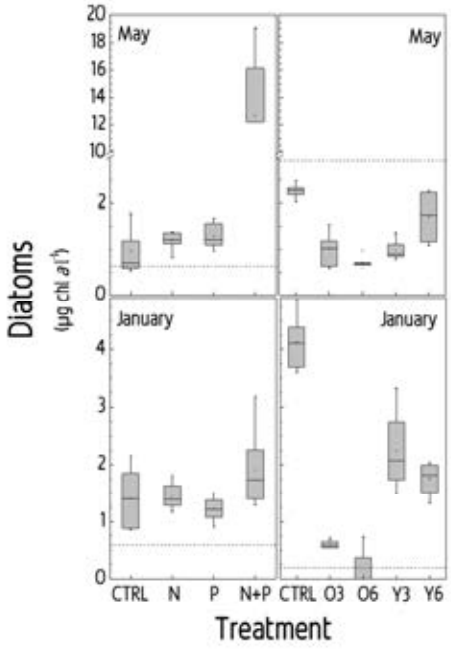

Figure IV.5. Boxplots of the nutrient and leaf bioassay results for cryptophytes. The horizontal dashed line indicates the initial concentrations (i.e., t_0).

Table 1. Results of means comparisons for each algal group and for both the nutrient and leaf bioassays. The letter designations refer to the different treatments and a continuous underline indicates the means were not significantly different ($p > 0.05$). The means are ordered from lowest to highest values. Non-significant treatment effects, based on the univariate ANOVAs, are symbolized by NS ($p > 0.01$).

Algal Group	Nutrient Bioassay	Leaf Bioassay
Chlorophyll *a*	P C N NP	O6 O3 Y3 Y6 C
Chlorophytes	C P N NP	O6 O3 Y3 C Y6
Cryptophytes	N P C NP	O6 O3 Y6 Y3 C
Cyanobacteria	P C N NP	O3 Y6 O6 Y3 C
Diatoms	C P N NP	O6 O3 Y3 Y6 C
Dinoflagellates	NS	NS
Haptophytes	C P N NP	O6 O3 Y6 Y3 C
Non-Peridinin Dinoflagellates	NS	Y3 C O6 Y6 O3

Nutrient Addition Bioassays

The phytoplankton community (total chl a) showed a significant positive response in the P additions only ($F=13.83$, $p < 0.001$) (Fig. II.A.1; Table 1). The majority of algal biomass in both January and May was attributed to diatoms, cyanobacteria, and dinoflagellates (Figs. II.A.2-II.A.5). The nutrient addition bioassay results were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and nutrient treatment as the second factor (control, N, P, NP). The multivariate tests indicated a significant nutrient effect (Pillai's trace = 0.991, $F = 3.45$, $p < 0.001$) and block effect (Pillai's trace = 0.852, $F = 38.63$, $p < 0.001$). Subsequent univariate ANOVAs showed nutrient addition effects on each individual group except dinoflagellates and non-peridinin dinoflagellates ($p < 0.01$). *A posteriori* multiple comparisons found significant positive effects of NP for total chl *a*, diatoms, chlorophytes, and haptophytes ($p < 0.05$) (Table 1).

Mangrove Leaf Bioassays

In the mangrove leaf bioassays, the phytoplankton community (chl *a*) had a negative response to the addition of leaves relative to the control ($F = 47.62$, $p < 0.001$) (Fig. 2; Table 1). Algal group responses to the mangrove leaf addition bioassays were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and leaf treatment as the second factor (control, 3 orange, 6 orange, 3 yellow, and 6 yellow leaves). The multivariate tests indicated a significant leaf addition effect (Pillai's trace = 2.091, $F = 5.95$, $p < 0.001$) and block effect (Pillai's trace = 0.822, $F = 23.05$, $p < 0.001$). Subsequent univariate ANOVAs indicated leaf addition negative effects on all groups except dinoflagellates ($p < 0.01$). The orange leaves had the highest negative impact on group abundances (Figs. 3-5; Table 1).

In order to test the ability of ChemTax to assign the correct relative abundances for dinoflagellates, peridinin concentration (a diagnostic photopigment for dinoflagellates) was plotted vs. dinoflagellate relative abundance (Fig. II.A.6). A least-squares linear regression found a high positive relationship between the two variables ($F = 3853$, $p < 0.01$; adj. $R^2 = 0.97$) and suggests that ChemTax accurately assigned dinoflagellate abundances.

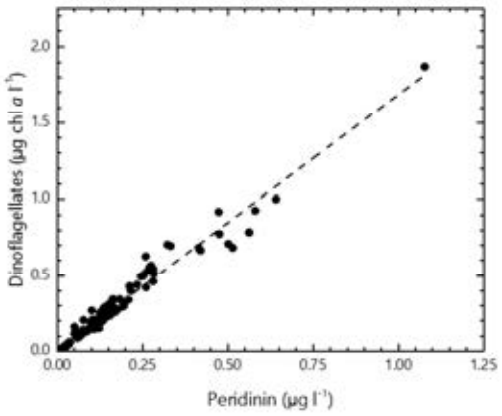

Figure IV.6. Scatterplot of dinoflagellate relative abundance vs. peridinin concentration. Peridinin is a diagnostic photopigment for dinoflagellates. The dashed line indicates the results of a least-squares linear regression ($F = 3853$, $p < 0.01$; adj. $r^2 = 0.97$).

DISCUSSION

The results of the nutrient addition bioassays suggest that the total phytoplankton community (chl *a*) as well as the chlorophytes, cryptophytes, cyanobacteria, diatoms and haptophytes were co-limited for N and P. The singular

additions of excess N or P in the bioassays forced conditions of N or P limitation for phytoplankton. Dinoflagellates, however, did not show a response to the nutrient additions and suggests that neither N, P, nor NP were limiting for growth. A comparison of initial and final dinoflagellate concentrations in the bioassays shows that the incubation period was sufficient to measure significant growth responses, but growth in the experimental treatments was not different from the controls. In contrast to other studies (Sellner et al. 2001, Fan et al. 2003, Badylak et al. 2004, Phlips et al. 2004, 2011), nutrient enrichment did not stimulate growth of dinoflagellates in this study. Our results are, however, consistent with the recent suggestion by Usup et al. (2012) that *P. bahamense* is not competitive and will not become dominant under high N and P conditions.

Dinoflagellate biomass in this study did not seem to be directly dependent on water column nutrient (nitrogen, phosphorus) concentrations. However, it is possible that *P. bahamense* and potentially other dinoflagellate species utilize benthic nutrient sources. Diel vertical migrations by dinoflagellates are not uncommon, and this behavior enables them to take up benthic nutrient sources rather than from the lower concentrations in the water column (Reynolds 2006). Additionally, the ability for internal storage of nutrients combined with possible use of dissolved organic phosphorus may explain the ability of dinoflagellates to persist in Mangrove Lagoon (Phlips et al. 2011, Usup et al. 2012).

Dissolved organic matter (DOM) provides an important nutritional source for dinoflagellates, and mangrove leaves leach a relatively large amount of labile DOM as well as being an extremely important source of sugars, protein, polyphenols, and inorganic nutrients to the surrounding waters (Benner et al. 1986, 1990a,b). Although DOM may provide a source of nutrition or nutrients for dinoflagellates, mangrove DOM may also be inhibitory to the growth of other phytoplankton (Doblin et al. 1999, Stolte et al. 2002). In our study, all phytoplankton groups except the dinoflagellates were negatively affected by the addition of mangrove leaves. Thus, DOM inputs via senescent mangrove leaves seem to have an inhibitory effect on all phytoplankton groups except the dinoflagellates. Collectively these results show phytoplankton community composition and abundances may be controlled by a balance between low nutrient concentrations and mangrove organic matter input in this lagoon. Under the ambient conditions of low nutrient and high DOM concentrations in the lagoon, dinoflagellates may have a small competitive advantage over other phytoplankters.

Other studies have suggested that the slow growth rates and high nutrient loading rates may preclude the growth of dinoflagellates (Tang 1996, Phlips et al., 2006, Usup et al. 2012). Conversely, the restricted or episodic supply of new inorganic nutrients, combined with large size and motility, may provide *P. bahamense* and other dinoflagellates with the ability to search for and store nutrients in more stable water columns (Phlips et al., 2006, Reynolds 2006, Usup et al. 2012). As with work conducted in similar systems, we suspect that long water residence time and calm conditions in Mangrove Lagoon further contribute to a favorable environment for dinoflagellate growth, including *P. bahamense* (Seliger et al. 1970, 1971, Margalef 1978, Sullivan & Swift, 2003, Greeney 2007, Phlips et al. 2011).

Although measuring toxin production was not a focus of the current study, it is worthwhile noting that some strains of *P. bahamense* produce a variety of paralytic shellfish toxins (PSTs). PST production by *P. bahamense* was previously assumed to be problematic in Southeast Asia (e.g., MacLean 1989), but recent work has linked production of the primary PST compound, saxitoxin (STX), with *P. bahamense* blooms in Florida's Indian River Lagoon (Phlips et al. 2004; Landsberg et al. 2006), a system that is similar to SARI by having long residence times but differs in the amount of surrounding human development and nutrient inputs. STX has also been detected in stranded manatees from the same region of Florida (Capper et al. 2013), suggesting that *P. bahamense* may pose an emerging wildlife and potentially public health threat in Atlantic waters. The extent to which nutrient form, concentration, and PST levels for *P. bahamense* may be associated are not clear, but laboratory studies have shown that cellular PST levels remain constant in *P. bahamense* cultures reared over a wide range of nitrate concentrations (Usup & Anderson 1996). However, the extent to which PST may vary with other nutrient forms and levels, including DOM, is an open question.

Bioassay incubation corral in Mangrove Lagoon

Pyrodinium bahamense cysts are found in Mangrove Lagoon sediments and peak at a depth of 8 – 24 cm, suggesting that dinoflagellates and bioluminescence have been a persistent feature of Mangrove Lagoon. However, comparisons with other bioluminescent lagoons in the region (Puerto Mosquito, Vieques, Puerto Rico; Laguna Grande, Puerto Rico) indicate that Mangrove Lagoon has relatively low concentrations of *P. bahamense* cysts and thereby low abundances of *P. bahamense* (Reidhaar 2013).

CONCLUSIONS

In this study, diatoms were the dominant algal group and dinoflagellates comprised only 10 – 20% of the total community in terms of relative abundance. Simultaneous increases in both N and P loading to the lagoon will likely foster the growth of diatoms while additions will have little direct impact on dinoflagellates. Our experimental results further suggest that the effects of simultaneous increases in both N and P loading will likely promote blooms of phytoplankton species other than dinoflagellates in the particular biobay studied here. Furthermore, the removal of mangroves from the perimeter of the lagoon may result in a decrease in leaf deposition and could also promote the growth of non-dinoflagellate phytoplankton through a reduction in the inhibitory effects of mangrove DOM loading. The longer term effects of changes in nutrient and/or mangrove DOM loading on phytoplankton interspecific competition are unknown.

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Objective V. Estimate water residence time in Mangrove Lagoon (Pinckney)

Previous studies have indicated that water residence time over weekly time scales is an important factor determining the accumulation of the dinoflagellate *Pyrodinium bahamense* in Caribbean estuaries (Seliger et al. 1970, Badylak et al. 2004, Phlips et al. 2006, Greeney 2007). Residence times for waters in Mangrove Lagoon were calculated based on volumetric estimates of lagoon surface area, lagoon volume, and tidal prism volumes. The calculations were as follows:

$$T_V = (A_L * T_R)/V_L$$

Where

T_V = percent tidal volume of Mangrove Lagoon
A_L = surface area of Mangrove Lagoon = 38,083 m^2
T_R = tidal range = 0.1 to 0.5 m
V_L = volume of water in Mangrove Lagoon = 89,263 m^3

Thus

$$\text{Residence time (days)} = 1 / T_V$$

These calculations assume a diurnal tide.

The above equations were used to calculate the residence times of water in Mangrove Lagoon over a range of tidal heights (0.1 to 0.5 m). The maximum tidal range for Christiansted Harbor (the nearest tidal station) is 0.4 m. Residence times range from 23.5 to 4.8 days, depending on the tidal range. Assuming a dinoflagellate growth rate of 0.5 d^{-1} (Seliger et al. 1971, Phlips et al. 2006), the dinoflagellate population would double every two days. Thus, the population could undergo two doublings at the shortest possible residence time. For comparison, the residence time for the Puerto Mosquito biobay was estimated to range between 6.8 and 9.6 days (Greeney 2007).

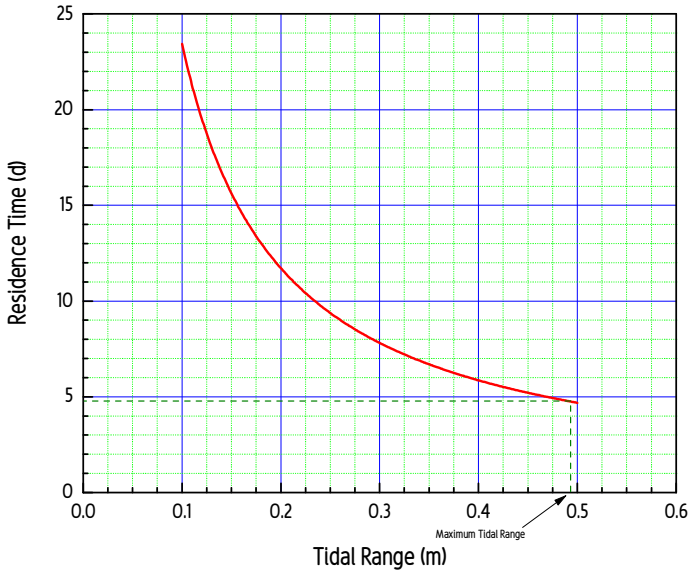

Figure V.1. Calculation of residence times as a function of tidal range for Mangrove Lagoon.

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Objective VI. Establish an outreach program to inform local citizens and ecotourism industry of research and findings (Castillo, Reale-Munroe, Taylor, Pinckney, Lane)

At the initiation of the project, the lead PI (Pinckney) and co-PIs (Castillo, Lane, Reale-Munroe and Taylor), with the assistance of the NPS, held a "town hall" meeting on January 10, 2013 at the NPS Salt River Visitor's Center and invited local tour operators, educators, and the general public. The purpose of this meeting was to inform the public about the project and solicit volunteers for the sampling efforts. A Mini-Symposium (24 May 2014) was held at UVI Great Hall which included student posters and presentations by PIs on project results to inform the public of the major research findings and recommendations for the management of Mangrove Lagoon.

There were 3 UVI undergraduate students (listed below) who were involved with the project and presented their projects at both local and national conferences (see below):

1. Lorne Joseph (Major: Chemistry) presented "Comparative Study of Nutrient and Chlorophyll Content in Salt River Bay and Bioluminescent Mangrove Lagoon, St. Croix, US Virgin Islands" at:
    a. UVI Research Day 2014, St. Thomas, VI
    b. Annual Biomedical Research Conference for Minority Students (ABRCMS) 2013, Nashville, TN
2. Gejae Jeffers (Major: Computer Science) presented "Vertical Chlorophyll Profiles in Salt River Bay and Bioluminescent Mangrove Lagoon, St. Croix, US Virgin Islands" at:
    a. UVI Research Day 2014, St. Croix, VI
    b. UVI Emerging Caribbean Scientists (ECS) Spring Research Symposium 2014, St. Croix, VI
    c. Annual Biomedical Research Conference for Minority Students (ABRCMS) 2013, Nashville, TN
    d. UVI Emerging Caribbean Scientists (ECS) Fall Research Symposium 2013, St. Thomas, VI
    e. UVI Emerging Caribbean Scientists (ECS) Summer Research Symposium 2013, St. Thomas, VI
3. Jamila Martin (Major: Applied Mathematics) presented "Comparative Study of Nutrient Content in Salt River Bay and Bioluminescent Mangrove Lagoon, St. Croix, US Virgin Islands" at:
    a. UVI Research Day 2014, St. Thomas, VI
    b. UVI Emerging Caribbean Scientists (ECS) Spring Research Symposium 2014, St. Croix, VI
    c. 2014 Emerging Researchers National (ERN) Conference in STEM. Washington, DC
    d. Ana G. Méndez University System (AGMUS) Conference 2013, San Juan, PR
    e. UVI Emerging Caribbean Scientists (ECS) Fall Research Symposium 2013, St. Thomas, VI
    f. UVI Emerging Caribbean Scientists (ECS) Summer Research Symposium 2013, St. Thomas, VI

We would like to also report that Jamila Martin won the Best Poster Presentation Award at the ECS Fall Research Symposium 2013 and at the 2014 ERN Conference in STEM. We were also invited to speak to high school students at the St. Croix Educational Complex last summer of 2013.

Recreational Experience

As the kayaks enter the narrow channel into Salt River bioluminescent bay they start to see more lighting up of their paddle's wake as they venture to the NE. Most kayak companies stop when they reach the area around station 5 and briefly discuss the bioluminescence and point out any larger forms such as comb jellies or fish wakes. They encourage the participants to generate their own movement so that they can see it clearly. There is a variety of ways that the different kayak companies run their tours. Some just sit relatively quietly while others talk more loudly and longer. Some companies "raft up" while some allow their participants to explore the Bay on their own for a period of time. Many companies only spend 15 minutes in the Bay while others stay longer. It seems like most companies make their way around the entire Bay before leaving.

The National Park Service has been collecting data from some of the kayak operators on their use of Salt River Estuary. There are at least 4 kayak companies regularly using the site; three of these have submitted use data for 2013. A graph of this data shows that in any given month 182 to 827 people are kayaking in the Estuary. Most of people participating in kayak tours are visitors to the island. St. Croix residents largely do not have the opportunity to enjoy the site. Because of this, relatively few residents have direct knowledge of the phenomena which occurs in the bioluminescent bay. The objective of this outreach component of the project was to increase awareness of the bioluminescent bay and allow high school students the opportunity to experience kayaking in the Bay at night.

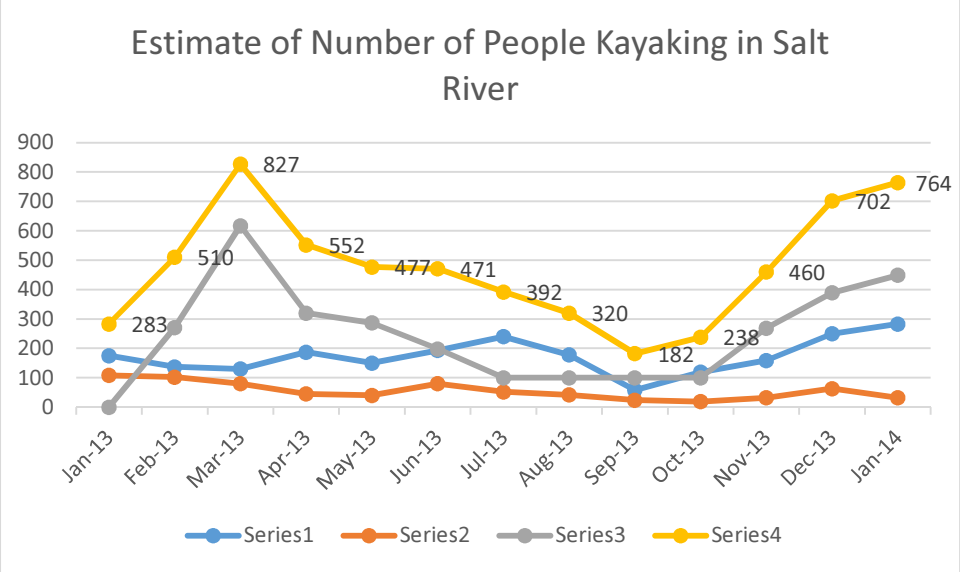

Fig. VI.1. Total number of kayakers visiting Mangrove Lagoon based on the survey of 3 tour operators.

Engaging the community in the amazing biological phenomenon in Salt River Bay is important to any decision made to develop the surrounding environments. The local community must understand the resources, the conditions under which it is occurring, and the potential impacts of any alterations. Through this project information on the

Bay and the work being done at the site was transmitted to local school students through presentations, an exhibit and kayak tours:

- Fifteen school (grades 8-12) presentations were made at local schools increasing student awareness of the Salt River bioluminescence and issues surrounding it. A total of 423 students participated in the classroom presentations at three high schools including St. Croix Educational Complex (142), Good Hope Country Day School (47), and Central High School (12).

- For classes that received the presentation, VIMAS arranged kayak tours through Anchor Dive so that students could experience the bioluminescence at the bay. These tours also included parents when requested. Fifteen kayak tours involving 201 students were made. Letters written after the tour showed that the students learned a great deal and very much enjoyed the tour.

- In addition, an exhibit explaining bioluminescence, where it occurs locally and what research is taking place at Salt River was showcased at the St. Croix annual EcoFair in April 2013 where a total of about 1000 students visited during the two-day event and 300 students directly heard a presentation on the project.

Through these outreach efforts over 700 students and parents were directly impacted by the program and are better informed about Salt River Bay, its past and the current NPS, GVI and partner actions to restore and preserve this unique natural area.

SUMMARY

The research described in this report was a multi-investigator, multi-disciplinary, and multi-institutional project designed to provide a more detailed description of the physical and biological features associated with the bioluminescence phenomenon that occurs in Salt River Bay NHP & EP's Mangrove Lagoon, St .Croix, Virgin Islands This study reported the definitive identification of the bioluminescent dinoflagellate species (*Pyrodinium bahamense* var. *bahamense*) and an "*Alexandrium*-like" species, relationships between water quality parameters and dinoflagellate (as well as all phytoplankton) concentrations, a determination of nutrient concentrations and which nutrients are potentially limiting the growth of dinoflagellates (and phytoplankton), the spatial distribution, abundance, and a chronology of previous blooms based on preserved cysts, and approximations of water residence time. Provisions were made for the involvement of stakeholders, students (elementary to college), educators, and the general public through a carefully designed and implemented outreach program. The final report provides essential baseline data for assessing future changes in water quality and bioluminescent dinoflagellates in Mangrove Lagoon.

The continuous monitor record (January – December 2013) showed a typical dry/wet season pattern, with fairly rapid reductions in salinity (1 – 5 psu) and increases in turbidity following most rainfall events. Nutrient (N and P) concentrations were generally low, less than 10 µm N and 2 µM P, during the study period and were highest during April – July. There was a notable increase in both N and P in late November. The majority of the bioavailable N was in the form of ammonium (NH_4^+) which suggests a benthic origin or regenerated N rather than runoff. Mixing events in the lagoon, due to storms or strong winds, may resuspend sediments and nutrients in the water column. The Redfield Ratio for DIN:P was always less than 16, which suggests N-limitation for phytoplankton growth. Subsequent nutrient addition bioassays in January and May showed a co-limitation of N and P. Light attenuation measurements suggest that there is sufficient sunlight to support photosynthesis by benthic microalgae and demersal phytoplankton. The waters of the lagoon appear to be homogeneous from top to bottom with respect to temperature, salinity, and dissolved O_2. A portion of the phytoplankton community (ca. 25%) undergoes migration from bottom to surface waters during nighttime and this behavior occurs over much of the lagoon.

The phytoplankton community in Mangrove Lagoon was composed primarily of diatoms, dinoflagellates, prymnesiophytes, and cyanobacteria. The relative contributions of each of these groups varied seasonally. An early summer diatom bloom was correlated with an increase in dissolved inorganic nitrogen (DIN) concentration while the late summer/fall blooms were not related to DIN concentrations or N:P ratios. However, the fall blooms seemed to co-occur or follow rain events. During the day, phytoplankton concentrations were higher in the bottom waters, possibly due to negative phototaxis in the shallow lagoon waters (< 4 m). We hypothesize that the dinoflagellates are utilizing benthic nutrients (i.e., from nutrient regeneration at the sediment water interface) for growth and are not dependent on water column nutrients. Another hypothesis is that the dinoflagellates, including *P. bahamense*,

are using alkaline phosphatase to process DOP as a source of P for growth and nutrition. Phytoplankton concentrations were higher during the late summer and fall months, with dinoflagellates composing as much as 90% of total phytoplankton biomass. Both variability and total biomass of phytoplankton was highest in the fall months (August-November).

The results of nutrient addition bioassays suggest that N and P were co-limiting for phytoplankton growth in January and May. The addition of mangrove organic matter (leaves) had a negative effect on all phytoplankton groups except the dinoflagellates. Simultaneous increases in both N and P loading to the lagoon will likely foster the growth of diatoms while nutrient additions will have little direct impact on dinoflagellates. Our experimental results further suggest that the effects of simultaneous increases in both N and P loading will likely promote blooms of phytoplankton species other than dinoflagellates in Mangrove Lagoon. Furthermore, the removal of mangroves from the perimeter of the lagoon may result in a decrease in leaf deposition and could also promote the growth of non-dinoflagellate phytoplankton (e.g., diatoms, cyanobacteria, prasinophytes, etc.) through a reduction in the apparent inhibitory effects of mangrove DOM loading. The longer term effects of changes in nutrient and/or mangrove DOM loading on phytoplankton interspecific competition are unknown.

Analyses of spatial and temporal variations in Mangrove Lagoon water chemistry, sediment geochemistry and *P. bahamense* cyst abundance indicated marked spatial heterogeneity in modern *P. bahamense* cyst concentrations and preferential habitats for the dinoflagellate along the northern and eastern shorelines. Temporal variations in *P. bahamense* cyst concentrations indicate that the dinoflagellate has not always been abundant in Mangrove Lagoon, given it was only connected to the bay in 1960s, and that currently populations may be on the decline. Based on the sediment analyses it appears that an open connection between Mangrove Lagoon and Salt River Bay along with a fringing mangrove community is likely important for sustaining high dinoflagellate and *P. bahamense* populations.

Estimates of water retention times range from 5 to 24 days, depending on the tidal range within the lagoon. This time is similar to residence times for other known biobays in the Caribbean (ca. 5 – 10 days). Given a doubling time of 2 days for dinoflagellates, water residence time is sufficient to allow substantial “blooms” of dinoflagellates as well as other phytoplankton groups such as diatoms and cyanobacteria that are common in Mangrove Lagoon.

The bioluminescence phenomenon in Mangrove Lagoon is a natural process that could be affected by a variety factors outside management controls. For example, hurricanes, drought, changes in sediment loading, water residence time, and excessive rainfall may result in the disappearance of *Pyrodinium* from Mangrove Lagoon. Similarly, natural cycles of phytoplankton abundance and competition, phytoplankton grazers, and nutrient loading could affect *Pyrodinium* abundances.

RECOMMENDATIONS

The short duration of this study (< 1 year) makes it difficult to draw robust conclusions about the interannual relationships between the physical, chemical, and biological characteristics and dinoflagellate bioluminescence in Mangrove Lagoon. Regular annual patterns cannot be discerned from ephemeral events. However, the results of this study can be used to generate some preliminary and limited recommendations for actions that may be necessary to guarantee the persistence of bioluminescence in Mangrove Lagoon.

1. The shallow sill accreting at the mouth of the lagoon will likely continue to restrict flow in and out of Mangrove Lagoon. The historical cyst data suggests that *P. bahamense* abundance is declining in the lagoon and this decline may be related to the reduction in water exchange with Salt River Bay. Maintenance dredging may be required to prevent further shallowing of the sill at the mouth of the lagoon and facilitate flushing of lagoon waters.

2. Phytoplankton production in the lagoon is nutrient limited with N or N and P co-limitation. Additions of N and P will likely foster diatom blooms. The effects of these blooms on dinoflagellate abundances are unknown. Steps should be taken to minimize nutrient inputs via runoff, groundwater, or from Salt River Bay. Buffer zones of native vegetation and storm water control should be considered in all future planning.

3. Mangrove organic matter, derived from the direct deposition of mangrove leaves or via runoff, may be important for suppressing the growth and proliferation of algal groups other than dinoflagellates. Mangroves along the margins of the lagoon should be protected from future reductions in coverage.

4. Water quality and phytoplankton monitoring at weekly intervals should be continued to document annual variations and to quantify possible changes resulting from activities in the adjacent watershed.

5. Attempts were made during this study to characterize the bacterial community and abundances. Limitations of on-site analytical instrumentation and personnel training prevented the collection of these data. These measurements are critical due to the important linkages between bacteria and water quality. Future efforts should seek funds for the purchase of needed instrumentation and training of personnel for on-site sample collections and processing. Necessary equipment would include a temperature controlled incubator, autoclave, research grade microcentrifuge (> 12,000 g), peristaltic pump, and ultra-cold freezer (-80° C).

6. Kayakers should not use any insect repellants, sunscreen, and other personal care products (as well as swimming in the water) to minimize exposure of toxic chemicals to sensitive phytoplankton.

7. Use of internal combustion engines should be restricted and tightly regulated within the waters of Mangrove Lagoon.

8. Future studies should continue to have a strong outreach/education component

9. Management decisions should be “ecosystem-based” and include the entire Salt River Basin because the lagoon is one small component in a much larger system.

10. Future topics for Mangrove Lagoon studies include:

    Hydrodynamic model of Mangrove Lagoon and Salt River Bay

    Groundwater inputs into Mangrove Lagoon

    Current measurements at the mouth of Mangrove Lagoon

    Monitoring the depth and migration of the sill at the mouth of Mangrove Lagoon

    Characterization of the microbial community including coliforms and human pathogens

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